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BIOLOGIC EFFECTS OF RADIOACTIVE PHOSPHORUS POISONING IN RATS*

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This report summarizes a study of the biologic and morphologic effects of radioactive phosphorus poisoning in rats. Interest centered on the types of lesions produced by this form of internal radiation, their progressive development, variation in distribution and severity, effects on the organism as a whole, reversibility or irreversibility, and the factors involved in causing death.

The isotope P^{32} has the advantages of ease of handling, wide distribution in body tissues, and short half-life (14.3 days). It is a pure beta ray emitter with average and peak energies of 0.695 and 1.69 mev., respectively. Since most of the energy from beta rays is absorbed within a few millimeters of tissue, this is the range of maximum damage. The doses of P^{32} employed in this study were in the lethal range.

It is essential to keep in mind that the effects of any internally administered radioactive isotope depend not only on the dosage, type of emission and half-life, but also on the distribution, *i.e.*, uptake by various tissues and organs, and the sensitivity of the tissues involved. The lesions produced by radiophosphorus should be interpreted in this light. It is well known that the hemopoietic and lymphoid systems, the gonads, and the intestinal tract are the most radiosensitive organs of the body. Thus while the uptake of P^{32} by liver exceeds considerably that by testis, the latter shows a much more severe lesion.

Adult white rats of the C. F. Wistar strain, almost all male, obtained from Carworth Farms, were used. The animals were kept in an air conditioned unit, permitting constant temperature and humidity. Injection of the radioactive material was by the intraperitoneal route.

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Distribution of P^{32}

Data were obtained on the distribution of P^{32} in the tissues and organs, also on the manner of excretion and rate of loss from the body. Sixteen rats were used, each receiving a single intraperitoneal dose of 1 μc. of P^{32} per gm. of body weight. Four rats were then sacrificed at each of the following intervals after injection: 1, 3, 7, and 14 days. Individual metabolism cages were utilized in order to obtain the total excreta up to the time of sacrifice.

Tissues and organs to be assayed were weighed wet, dried at 85° to 100° C. for 48 hours, and then ashed at 800° C. Samples of bone were dissolved in concentrated nitric acid and dried. The intestinal contents were added to the excreta for assay. To simplify the calculations, the results were compared with a standard made from the original solution immediately following the injections.

The average uptake of P^{32} for various tissues and organs, expressed as a percentage of the injected dose per gram of tissue, is shown in Table I. Although P^{32} is a bone-seeking element, substantial deposit

Table I

Average Percentage Uptake of P^{32} per Gram of Tissue

Organ	Interval after injection			
	1 day	3 days	7 days	14 days
	%	%	%	%
Femur	4.75	4.52	4.35	3.60
Marrow	2.65	1.47	0.80	0.60
Vertebra	2.50	3.46	2.08	2.51
Thymus	1.34	1.04	0.60	0.37
Liver	1.20	0.64	0.17	0.24
Spleen	1.05	0.70	0.25	0.28
Pancreas	0.95	0.87	0.46	0.56
Lymph nodes	0.91	0.85	0.15	0.50
Adrenals	0.90	0.86	0.35	0.46
Small intestine	0.77	0.43	0.14	0.14
Kidneys	0.69	0.42	0.16	0.22
Urinary bladder	0.68	0.68	0.14	0.19
Large intestine	0.61	0.48	0.12	0.21
Stomach	0.57	0.49	0.08	0.22
Thyroid gland	0.56	0.39	0.26	0.17
Lung	0.56	0.44	0.12	0.18
Heart	0.55	0.41	0.18	0.23
Muscle	0.43	0.50	0.24	0.36
Gonads	0.31	0.37	0.18	0.20
Skin	0.27	0.23	0.07	0.10
Blood	0.22	0.13	0.12	0.06
Brain	0.10	0.14	0.16	0.16

occurred in such organs as liver, thymus, spleen, pancreas, and adrenals. Table II shows the average percentage of the total dose retained by the skeleton and by soft tissues and viscera, the percentage excreted up to the time of sacrifice, and the total amount of P^{32} recovered. Almost all of the P^{32} given was recovered at the various time intervals. Uptake

by the skeleton increased during the first 3 days after injection and then declined over the next 4 days. Distribution in the soft tissues and viscera was maximum at 24 hours and then dropped progressively until the end of the first week. Thereafter the amount of activity in these sites and in bone was stabilized. Since the radioactive material in soft

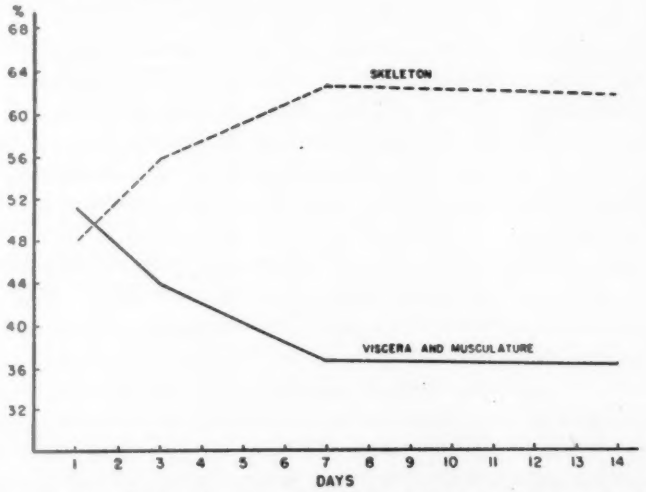
TABLE II

Average Percentage of Total Dose of P^{32} Recovered after Various Intervals

	1 day	3 days	7 days	14 days
	%	%	%	%
In soft tissues and organs	38.44	35.63	19.98	20.63
In skeleton*	35.56	45.44	34.00	34.18
In excreta	22.72	24.44	45.33	48.76
Total percentage recovered	96.72	105.51	99.31	103.57

* The figures for the entire skeleton were obtained by multiplying the results for the femur by 25.

tissues and viscera was in a more labile state, these sites served as the main source of excretion of P^{32}. Text-figure 1 shows the proportion of the total retained dose in the skeleton and in the soft tissues and viscera during the first 2 weeks.



Text-figure 1. Percentage of total dose of P^{32} retained in the skeleton and in the soft tissues during 14 days after administration.

A rough index of the distribution of P^{32} in the body was obtained also by means of radioautograms. These were prepared by placing radioactive tissues in close contact with photographic emulsion, such as x-ray film, and then developing the film after proper exposure.[1-6] Regions of darkening in the films represent areas of accumulation of the radioactive material. Figure 2 consists of autograms prepared from

paraffin sections (15 μ thick) of a rat injected with 4.5 μc. of P^{32} per gm. and sacrificed 3 days later. Considerable activity is present in bone, liver, spleen, and lymph nodes, and only minute activity in brain, lungs, and testes. Figure 1 shows diffuse activity in the femur of a rat sacrificed 3 days after administration of 10 μc. of P^{32} per gm.

Whole body radiograms, made at intervals of 1 to 168 hours after the injection of 200 μc. of P^{32} by placing the flat surface of hemisected frozen rats on x-ray film, are shown in Figure 3. The localization of the P^{32} can be determined by comparing the photographs of the animals with the x-ray films. There was diffuse distribution of radioactive material during the first 6 hours and subsequently a diminishing concentration in the soft tissues. At 1 week almost all of the activity appeared to be in the skeleton.

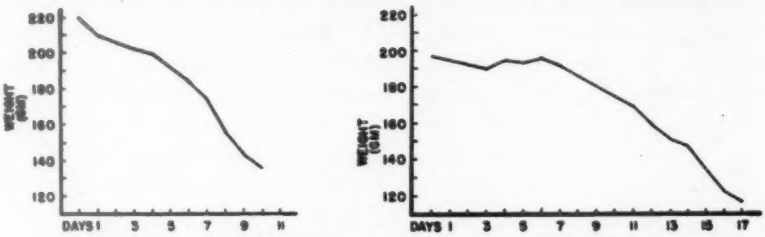
Data on Rats Given an LD_{50} Dose of P^{32}

In this laboratory the LD_{50} dose of P^{32} for the rat was originally determined to be 4.5 μc. per gm. of body weight. Subsequently, the mortality with this amount showed considerable variation, from 25 to 100 per cent. Of a total of 101 animals injected, 71 have died and 30 recovered. The majority of the rats with a fatal outcome were dead within 2 weeks after the radioactive substance was injected and, with a few exceptions, all died within 3 weeks. Thus, while referred to as an LD_{50}, the dose was actually equivalent to a 20 day LD_{70}. A possible explanation is that the P^{32} supplied later from Oak Ridge may have contained less carrier, so that more of the phosphorus was utilized and less excreted.

The administration of P^{32} was followed by a latent period lasting from a few days to 1 week, during which the animals appeared to be in good condition. Then they developed progressive apathy, weakness, and pallor. There was ruffing of the fur. Food and water were usually refused. From about 1 week on, hemorrhagic crusts were present about the eyes and nose. Death occurred in what appeared to be a state of profound cachexia and inanition. Marked loss of weight was observed in all animals which died. The usual pattern was a slight daily decrease during the first week after injection of P^{32}, followed by a more pronounced daily reduction over the next 3 to 7 days until death (Text-fig. 2A). In some rats, body weight was maintained for the first few days and showed a continuous decline thereafter (Text-fig. 2B). At the time of death, loss of weight ranged from 20 to 40 per cent of the initial control weight.

Thirty of the 101 animals recovered. The reaction to the radioactive material varied in this group. Objectively, some rats remained well

and either maintained weight or showed only slight loss. Others had moderate weight loss (up to 20 per cent) during the first week or two, accompanied by apathy, anorexia, and weakness, and occasionally by slight hemorrhage about the nose. The weight then rose progressively and returned practically to normal after 3 or 4 weeks. Animals which



Text-figure 2, A and B. Typical weight curves in rats killed by P^{32}.

recovered were sacrificed and autopsied at various intervals in order to obtain information on the phase of repair and reversibility of the pathologic changes. Observations extended over a period of 1 year.

Hematologic Studies

Hematologic studies were performed on 15 rats which died following administration of an LD_{50} dose of P^{32}. Control values were established for 3 successive days prior to injection. Red and white blood cell and platelet counts were made 16 and 24 hours after injection and at daily intervals thereafter until death.

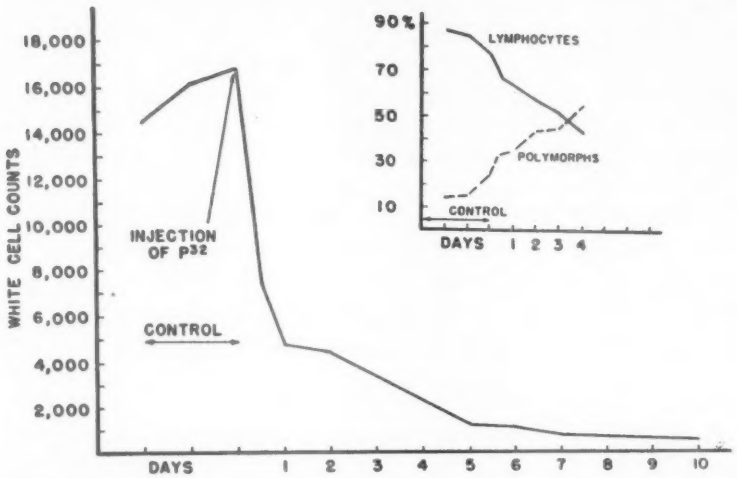
The results were in accord with previous accounts of radiation effects.[7-12] All formed elements of the blood were reduced, resulting in aplastic anemia. The white blood cells showed a rapid and progressive decline (Text-fig. 3). At 16 hours the counts often were lowered to one-half the control values. Marked leukopenia of a few thousand cells was present 24 to 48 hours after the P^{32} was given and the level was generally below 1000 cells after 5 days. A few days before death the counts were extremely low, *i.e.*, about 200 to 400 cells per cmm.

Differential smears were studied for the first 4 days (Text-fig. 3). These revealed a reduction in lymphocytes from the normal of about 80 per cent to 50 or 60 per cent as early as 16 to 24 hours and a continuous decline thereafter. Conversely, polymorphonuclear leukocytes showed a relative increase. Since the total white blood cell counts were very low, both granulocytes and lymphocytes were markedly reduced in absolute number.

Red blood cell counts remained at or near the normal levels for the first 4 to 6 days after injection of the P^{32}. Within the next few days and continuing until death, there was a persistent and fairly

rapid decline to anemic levels of about 4 to 5.5 million cells per cmm. (Text-figure 4).

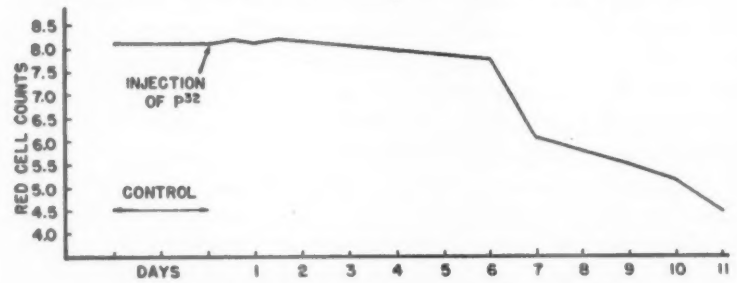
Blood platelets were counted by the method of Fonio.[13] The range of the control values was from 600,000 to 1 million per cmm. The levels remained essentially unchanged for the first 4 or 5 days and



Text-figure 3. White blood cell and differential counts in rats killed by P^{32}. The unnumbered days indicate the control period before injection.

fell progressively thereafter. At death, *i.e.*, from 7 to 21 days after the P^{32} was given, the counts ranged from practically zero to several thousand per cmm.

Rats which survived the LD_{50} dose showed similar but less marked hematologic changes. The leukopenia was less severe, reaching levels



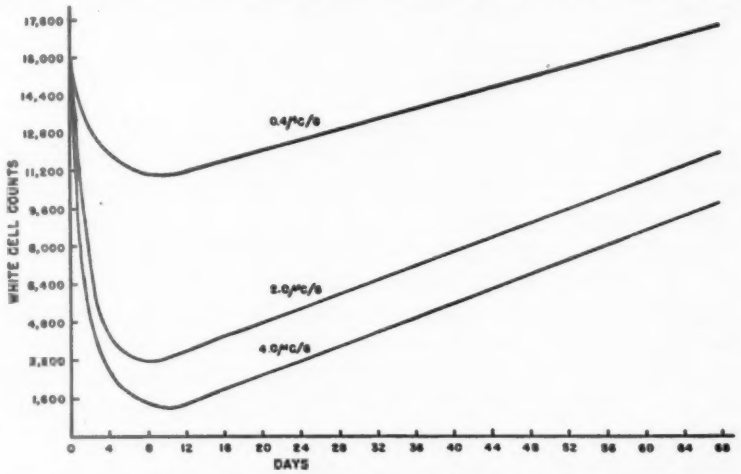
Text-figure 4. Red blood cell counts in rats killed by P^{32}.

of 4000 to 5000 cells. Between the first and second weeks after injection the red cells fell to about 4 million per cmm. and the platelets to 100,000. Only a few rats were followed subsequently for recovery.

After 2 or 3 months the red cell counts were normal and the white cell and platelet counts had risen nearly to low normal levels.

A supplementary study was made of 6 rats in which white cell counts were made at 30 minute intervals over a period of 6 hours following an LD_{50} dose of P^{32}. They showed an average elevation of 4000 cells per cmm. over the controls from 3 to 5 hours after the P^{32} was given and declined thereafter. This is in accord with the observation of early leukocytosis following radiation.[8,14,15] The rise is mainly polymorphonuclear (both polymorphonuclear and lymphocytic with lower doses) and according to Bloom and Jacobson [16] is due to mobilization rather than to new formation of cells.

Serial white blood cell counts were performed on 3 groups of rats injected with 0.4, 2.0, and 4.0 μc. of P^{32} per gm., respectively. There were 10 animals in each group. The results are shown in Text-figure 5.



Text-figure 5. White blood cell counts following injection of various doses of P^{32}.

The white blood cells fell rapidly within the first few days after injection, reaching their lowest level at about 8 to 10 days, and the reduction was roughly proportional to the dose. Thereafter a progressive rise occurred with complete or substantial recovery after 2 months depending on the amount injected.

Gross Findings in Rats Which Died Following P^{32} Poisoning (LD_{50} Dose)

The animals which died following P^{32} poisoning (LD_{50} dose) were markedly emaciated and showed depletion of fat. Significant gross changes were confined essentially to the hemopoietic tissues. Heart,

liver, kidneys, adrenals, and brain revealed no abnormality. There was generally no gross evidence of infection in the mouth, pharynx, and nasorespiratory or intestinal tracts. The bone marrow was dark red and of thin, watery or gelatinous consistency. The lymph nodes and spleen were reduced in size, the latter often considerably. The nodes were frequently red or grayish red. Thymus was uniformly much smaller than normal, sometimes tiny and difficult to identify. Although weights were not recorded, the testes did not appear to be significantly reduced in size. Petechiae were observed fairly frequently on the outer surface. Internal hemorrhage was not conspicuous. However, petechiae of pleura and of gastro-intestinal mucosa were common. The contents of the stomach or bowel, especially cecum, occasionally were bloody or blood-tinged. No ulcers were found in the intestine, except for one animal with extensive necrosis and ulceration of the cecum.

Microscopic Lesions in Rats Which Died Following P^{32} Poisoning (LD_{50} Dose)

Significant microscopic lesions were present in the lymphoid tissues of rats which died following P^{32} poisoning (LD_{50} dose), including lymph nodes, spleen, and thymus. Bone marrow, intestines, and testes also were significantly affected.

Lymph nodes, spleen, and thymus were markedly atrophic and severely depleted of cells. Follicles, especially germinal centers, either were eliminated entirely or reduced in size. The remaining lymphocytes showed pyknosis, deformity, and often karyorrhexis. Mitotic activity was suppressed. The stroma was condensed, prominent, and often the seat of widespread hyperemia. Hemosiderosis was conspicuous in the spleen, usually less prominent in lymph nodes, and slight or absent in the thymus.

The bone marrow (sternum, vertebrae, and femur) was the seat of marked aplasia, showing depletion of all types of hemopoietic cells. The marrow usually was fatty and often revealed diffuse hyperemia and hemorrhage. Small scattered groups of hemopoietic cells could still be identified and these showed pyknosis and fragmentation of nuclei, also bizarre nuclear forms. Mitosis was depressed but not entirely eliminated. There was slight to moderate hemosiderosis.

The lesions of the intestine were confined essentially to the small bowel. Damage was limited to the epithelial cells of the crypts of Lieberkühn which showed pyknosis and slight nuclear fragmentation. However, many nuclei were swollen, vesicular, and prominent. It was plain that by the time of death the peak of damage had passed and

repair was well under way or even advanced. Most glands were partly or completely relined by new epithelial cells and many mitotic figures were in evidence. The lymphoid stroma of the intestine showed cellular depletion and degenerative change, but this was less severe than in the lymph nodes.

Lesions were present in the testes of all rats dead from 9 to 18 days after injection of an LD_{50} dose of P^{32}. There was loss of orderly arrangement of cells in layers, *i.e*, spermatogonia frequently were detached from the basement membrane and displaced toward the tubular lumen and spermatocytes released into the lumen. Spermatogonia and spermatocytes were most severely affected, the former, especially, were often depleted. Pyknosis was present, also irregular clumping of chromatin and diminution of mitosis, while lysis was less conspicuous. Karyorrhexis was rare and no significant amount of cellular débris was observed. Spermatogenesis was depressed but not arrested and activity was probably derived from the less injured spermatocytes. In some rats there was fusion of spermatocytes to form bizarre multinuclear giant cells. Spermatids, as well as cells of Sertoli and interstitial cells, showed no significant change. The usual compact arrangement of tubules was replaced by a loose structure, probably due in large part to interstitial edema, hyperemia, and hemorrhage. Of interest was the variation in severity of the lesion among rats which died at the same time interval, and even in adjacent tubules of the same rat. This was especially true of depletion of spermatogonia. No distinct regenerative activity was noted in the sections. The lining cells of the tubules of the epididymis revealed no abnormality. However, the lumina frequently contained moderate or large numbers of necrotic epithelial cells, probably derived from the testis.

The lesions in other organs and tissues were slight or insignificant and could not be attributed directly to radiation.

Development and Repair of the Lesions Produced by P^{32}

Evolution of the pathologic changes resulting from doses of 4.5 μc. of P^{32} per gm. was studied by sacrificing rats at intervals after injection, *i.e.*, 1, 6, and 12 hours and 1, 2, 3, 5, 7, and 10 days. Animals which survived were subsequently sacrificed at regular weekly and monthly intervals up to 1 year to determine the progress of regeneration. The following data afford a summary of the significant observations.

Bone Marrow. Routine sections from sternum, vertebrae, and shaft of the femur showed essentially similar lesions. Cell damage was observed as early as 1 hour after administration of P^{32} and was well

developed at 12 to 24 hours (Figs. 4 and 5). There were changes in hemopoietic cells of all types, especially the less mature forms. The nuclei were shrunken, pyknotic, irregular in shape, often distorted, and sometimes fragmented with separation or extracellular dispersion of chromatin particles. Lysis of nuclei was much less prominent. Staining property of the cells was altered, the cytoplasm contracted, and the granules of the myeloid cells indistinct or absent. Depression of mitotic activity was evident at 6 to 12 hours and persisted thereafter except for a rare or occasional mitotic figure.

Whether erythroblasts or the more primitive white blood cells, *i.e.*, myeloblasts, promyelocytes, and myelocytes, showed greater radiation damage was uncertain. Both types were extremely vulnerable and almost depleted after a few days. More mature myeloid cells such as juveniles, banded forms, and segmented polymorphonuclear cells were the least affected and these formed the bulk of the cells remaining in the marrow from 1 to 5 days after administration of the P^{32}.

At 1 hour the megakaryocytes were reduced in size, the cytoplasm homogeneous and acidophilic, and the nuclei hyperchromatic and irregular. These cells then underwent gradual shrinkage and dissolution and were virtually eliminated by the end of 1 week.

The marrow showed progressive hypoplasia. One hour after injection of P^{32} the texture of the tissues was looser than is normal, there was slight hyperemia, and also an occasional fat cell. Thereafter, fatty replacement was progressive and associated with marked hyperemia (Fig. 6). Hemorrhage was observed at 3 days and was often severe at 7 days. Depletion of hemopoietic cells usually reached a peak at 7 to 10 days when the marrow was essentially fatty or diffusely hemorrhagic (Fig. 7). Well preserved endothelial and reticulum cells, also fibrocytes, were present in the stroma and there was usually slight to moderate phagocytosis of hemosiderin pigment. Foci of persistent or perhaps delayed cellular necrosis were found (Figs. 8 and 9). Even in the presence of marked hypoplasia or virtual aplasia it generally was possible to identify a few scattered groups of hemopoietic cells, of both the red and white blood cell series, either partly or fairly well preserved, also a rare shrunken megakaryocyte. Presumably these served as a source of regenerative activity in animals which recovered.

Beginning regeneration was observed at about the tenth day in small, widely dispersed foci (Fig. 10). The latter comprised myelocytes, erythroblasts, and a few megakaryocytes, and showed mitotic activity. In general, repair was minimal until after 3 weeks, when expanding groups of cells began to replace the fat (Fig. 11). By 2 to 3 months the process was advanced and the tissue well repopulated

with all types of hemopoietic cells (Fig 12). Atypical hyperplasia was not observed.

The marrow of almost all animals sacrificed from 6 months to 1 year showed excellent regeneration. The gross appearance was normal or nearly normal. Hemopoietic cells were abundant and the fat content varied from occasional cells to about one-third the total cellularity. Only one animal of a group of 19 revealed persistent aplasia and a marked lag in repair.

Lymph Nodes. The administration of P^{32} resulted in hypoplasia of the lymph nodes. The change varied from moderate to marked, not only in rats studied at the same time interval but even in adjacently situated lymph nodes of the same animal. This probably was due to difference in uptake of the radioactive material. Cellular damage was evident at 1 hour and generally marked at 6 hours (Figs. 13 and 14). Practically all lymphocytes in cortex and medulla showed shrinkage and pyknosis of nuclei, often with deformity, irregular lobation, and fragmentation. The last occurred both in the germinal centers of the follicles and in the medullary cords, and was accompanied by phagocytosis of dispersed nuclear particles. Phagocytic activity was conspicuous during the first day and less prominent thereafter. However, macrophagic ingestion as well as lymphocytic degeneration and necrosis could be observed in some degree up to 2 weeks after injection of P^{32} (Figs. 15 and 16).

There was distinct suppression or arrest of mitosis after 1 hour. Cellular depletion of the cortex was rapid and in many instances most of the germinal centers were eliminated within 24 hours. Depletion of the medulla was usually less complete and loosely disposed groups of degenerated pyknotic cells persisted, especially in perivascular position. After a few days many cells, morphologically of plasma cell type, appeared in the medulla. Depletion of lymphocytes led to increasing prominence of the connective tissue stroma and reticulum cells, also of the vascular bed (Fig. 17). In some instances hyperemia was marked and the nodes consisted essentially of large lakes of blood. Hemosiderosis was common after several days and sometimes extensive.

Beginning repair was noted in a few instances at 3 or 5 days and was present in most rats after 1 week (Fig. 18). However, in some animals, apparently those with severe radiation effect, the process was delayed up to 2 or even 3 weeks. Regeneration was indicated by proliferating and expanding groups of immature lymphocytes in both medulla and germinal centers of the follicles (Figs. 19 and 20). There was variation in degree among rats studied at the same time interval, in different nodes of the same animal, and even in different parts of

the same node. By 2 months, reconstitution was well advanced, although still incomplete. After 3 months, the nodes usually were average in size or slightly enlarged. From 6 months on, recovery was good or excellent. In most instances there was virtually complete repopulation of both cortex and medulla. However, in the nodes of a few animals, persistent foci of depletion and retarded repair were observed within the cortex.

Spleen. The spleen showed marked destruction and depletion of lymphoid cells in both red and white pulp. Cellular lesions, present at 1 hour and pronounced at 6 hours, consisted of diffuse pyknosis, shrinkage, and deformity of nuclei, followed by karyorrhexis with dispersion and subsequent phagocytosis of chromatin particles (Fig. 21). Karyolysis was not prominent. Mitotic activity was reduced at 1 hour and plainly suppressed thereafter, although not entirely eliminated. An occasional mitotic figure could be found at most time intervals. After 12 to 24 hours, rhexis was less conspicuous and by this time most of the necrotic débris had been removed.

Erythroblasts and myelocytes, comprising extramedullary hemopoietic foci, showed similar damage and often were eliminated after 24 hours. The myelocytes seemed to disappear more rapidly and completely, but this could not be determined with certainty. In some instances occasional erythroblasts persisted during the entire course of the lesion. Megakaryocytes showed cell damage at 1 hour and then progressive shrinkage, often with virtual disappearance after the fifth or seventh day.

The degree of cellular depletion varied from moderate to severe. There often was marked depopulation of the red pulp within 12 hours. The germinal centers of the follicles shrank progressively and virtually were obliterated by 24 hours, leaving perivascular collars of pyknotic lymphocytes around the arterioles (Figs. 22 and 23). The stroma and vascular bed of the organ, also the reticulum cell network, became prominent after 6 hours and usually remained so for about 2 weeks (Fig. 24). After the fifth day there were numerous large macrophages with abundant cytoplasm filled with iron-containing pigment (Fig. 25).

Many spleens showed a long period of persistent depletion lasting from 10 days to 2 weeks, with no significant activity either in follicles or red pulp. However, regeneration was observed in some animals as early as 3 to 5 days after administration of P^{32}, was frequent at the tenth day and practically constant after 2 weeks (Figs. 26 and 27). Often there was fairly abundant repair in rats which had died within 2 weeks. Recovery was characterized by the appearance of immature lymphocytes showing mitotic figures, and at this stage proceeded more

rapidly in the red pulp than in the follicles. Erythroblastic regeneration appeared to precede that of the myelocytes. The megakaryocytes reappeared, enlarged, and became more numerous.

At 2 and 3 months after injection of P^{32}, repair was well advanced in spite of a lag in some organs in either red or white pulp (Fig. 28). However, even at this time there still remained much cellular depletion with prominence of vascular bed and stroma. In many instances myeloid activity in the red pulp exceeded lymphoid activity. Other animals showed pronounced reticulum cell hyperplasia of the red pulp.

In animals sacrificed from 6 months to 1 year, it was evident that over-all repair in the spleen was less effective than that in bone marrow and lymph nodes. Moreover, there was a distinct contrast between the red and white pulp. As a rule, the white pulp showed active or pronounced regeneration and consisted of large follicles with hyperplastic germinal centers, while the red pulp showed only relatively small groups of proliferating and expanding lymphocytes and a prominent condensed fibrous stroma. This was true even at 1 year after the single dose of P^{32} was injected. However, in 2 animals studied at 7 and 9 months, respectively, the appearance was reversed. No repair of follicles was present although there was fairly abundant regeneration of lymphocytes in the red pulp. In one rat which died 7 months after injection of P^{32}, there was still almost complete aplasia with no significant repair in either red or white pulp.

Thymus. The thymus responded to administration of radiophosphorus with rapid, progressive involution. Depletion of lymphocytes was well advanced at 1 hour, marked at 2 or 3 days, and virtually complete after 1 week (Figs. 29 and 30). Both cortex and medulla were depopulated, the former to a greater degree, especially in the later phase of contraction. In most animals dead within 12 days, the organ was tiny and consisted mainly of a condensed connective tissue framework with prominent fibrocytes and histiocytes and regions of hyperemia and sparse hemorrhage. Well preserved cords of epithelial cells were observed in several instances during the first week.

Degeneration and necrosis of lymphocytes were present at 1 hour and usually were well marked within 24 hours. Both cortex and medulla showed pyknosis. Karyorrhexis, of varying degree and sometimes marked, was the chief evidence of cell death and often there were numerous extracellular chromatin particles, either free or phagocytized by macrophages. Although generally prominent during the first day after P^{32} was given, fragmentation of nuclei was less conspicuous thereafter (Fig. 31). Karyolysis was observed infrequently. Mitotic activity was suppressed apparently almost from the start, although a rare

mitotic figure could be identified in rats sacrificed within 1 week. Phagocytosis of iron-containing pigment was minimal or absent.

The pattern of repair in the thymus was variable. Repopulation with lymphocytes was noted as early as 3 days after injection of the radioactive material and was more constant after 2 weeks (Figs. 32 and 33). The process began in the medulla and proceeded outward into the cortex. In some rats the organ, although moderately reduced in size, was fairly well reconstituted at the end of 1 or 2 months. In most animals, studied from 1 month on, the thymus was small, indicating persistent atrophy, or consisted of a tiny organ difficult to identify. However, microscopic sections of the mediastinal fat revealed thymic lobules which were considerably reduced in size, but well repopulated with lymphocytes in both cortex and medulla (Fig. 34). The thymus of several animals studied from 6 months to 1 year after P^{32} was injected showed virtually no repair and consisted mainly of condensed stroma.

Stomach and Intestines. The LD_{50} dose of P^{32} uniformly produced lesions of the gastro-intestinal tract, most marked in the small bowel and less severe in large intestine and stomach.

In the small bowel the main site of damage was in the crypts of Lieberkühn, while the cells lining the villi usually showed no significant alteration. Degeneration and necrosis of epithelium were present at 1 hour and reached a maximum at about 12 hours after the radioactive material was administered (Fig. 35). The nuclei were shrunken, pyknotic, and frequently the seat of rhexis, with scattering of chromatin fragments into the glandular lumina. However, many nuclei were enlarged, swollen, and vesicular. Karyolysis was infrequent. Mitosis apparently was depressed for the first 12 or 24 hours, although not eliminated entirely; many mitotic figures were distorted and probably represented arrested forms present prior to the injection of P^{32}. Cellular débris, although less evident after 24 hours, persisted in some instances for 10 days. During this period the crypts were partly filled with cells showing large, prominent vesicular nuclei. Repair, indicated by proliferating epithelium, became evident at 2 or 3 days, was well advanced by 7 or 10 days, and virtually complete at 2 or 3 weeks (Fig. 36).

The lesions in the colon were of similar type but less severe. Slight necrosis of epithelial cells and suppression of mitosis were observed during the first day. Thereafter, débris was rapidly eliminated and mitosis resumed, the latter becoming prominent by the third day. There was excessive secretion of mucus during the first week, associated with dila-

tation of the crypts. The lesions generally subsided after the fifth day and by 10 days the structure had returned essentially to normal.

Changes in the stomach were slight. There was involvement of both zymogenic (chief) and parietal cells, especially the latter. The zymogenic cells showed a minimal and transient lesion during the first 6 or 12 hours, *i.e.*, slight pyknosis and coarse granularity of the cytoplasm, with return to normal by 24 hours. In parietal cells, pyknosis, shrinkage, and irregularity of nuclei were distinct, also swelling and slight vacuolization of cytoplasm with retention of granularity. These changes often cleared within 1 or 2 days but occasionally persisted for about 5 days. By the seventh to tenth day after injection of P^{32}, the cells were practically normal.

Testes. Development of the testicular lesions produced by P^{32} was difficult to follow because of the variable damage at different time intervals. In many instances no significant progress could be detected between the first and tenth day after the radioactive material was given. However, on the whole, there appeared to be progressive reduction in the number of spermatogonia and probable diminution in spermatogenesis. Interstitial hyperemia and edema, interruption of orderly cell arrangement, and degenerative changes in spermatogonia and spermatocytes were present as early as 1 hour and were distinct at 24 hours (Fig. 37). At this time many tubules showed slight to moderate depletion of spermatogonia. Cellular débris was minimal.

From 2 to 4 weeks after injection there were progressive depletion of cells (Fig. 38) and depression of spermatogenesis. After 2 or 3 months, most testes were reduced in size and microscopically showed tubular atrophy with elimination in some instances of all but sustentacular cells (Figs. 39 and 40). The organs studied from 6 months to 1 year after injection of P^{32} were uniformly small, varying from one-half to one-fourth the expected size, and presented a flabby, watery yellow cut surface. Microscopically, atrophy varied from moderate to complete. In 14 of the 19 rats studied over this period, at least three-fourths of the tubules were essentially fibrous. In 5 rats, one-half or more of the tubules were cellular and some degree of spermatogenesis was noted. However, the arrangement of cells was often disorderly and the layer of spermatogonia only partly restored. Regeneration of the latter could not be followed clearly. It was difficult or impossible to distinguish true regeneration from resumption of activity by cells which were injured but not destroyed by radiation. One possible source of new spermatogonia was the numerous large, round, or irregular cells with prominent vesicular nuclei which appeared along the basement

membrane, but conversion of these to spermatogonia could not be traced.

Ovaries. Material from the ovaries was available on only 10 rats injected with an LD_{50} dose of P^{32}. Three animals died from 10 to 14 days after injection while 7 survived and were sacrificed from 7 to 8 months later. In the former the follicles were the seat of considerable pyknosis and rhexis of the lining cells, and nuclear débris was found within the lumina. Primary ova either were fairly well preserved or showed nuclear degeneration in the form of coarsely condensed chromatin. Most young ova revealed shrunken, poorly defined, pale-staining nuclei with atypical chromatin network and some were in a state of partial dissolution (Fig. 41). Corpora lutea and stroma were not significantly altered.

The ovaries of rats sacrificed after 7 or 8 months were the seat of advanced atrophy. The interstitial tissue was condensed and the vascular bed prominent (Fig. 42). Focal deposit of yellowish brown pigment was observed within the interstitial cells. The stroma often revealed collections of large lutein-like cells with abundant foamy cytoplasm and small nuclei (Fig. 43). Corpora lutea were not significantly altered. The follicles were reduced in number and size and were essentially anovular. A few primary ova still were present and showed slight to moderate degenerative change. Young ova were rare and the cells were shrunken, indistinct in outline, and had partially dissolved nuclei with coarsely clumped chromatin (Fig. 44).

Course of Events with a Lethal Dose of P^{32}

Injection of an LD_{50} dose of P^{32} is followed by a latent asymptomatic period lasting from 3 or 4 days to 1 week. During this time the rats seem well and often maintain their weight. However, regardless of the appearance of well being, within 12 hours after injection there is intensive destruction of tissue, principally in the hemopoietic organs. This is characterized by shrinkage, pyknosis, deformity, and fragmentation of nuclei, followed by extracellular dispersion and phagocytosis of chromatin particles. Mitotic activity is suppressed or arrested. By 24 hours, lymph nodes, spleen, and thymus usually are severely involuted. In the bone marrow all types of hemopoietic cells are damaged or destroyed, hemopoietic activity ceases, and there is marked aplasia within 3 to 5 days. The peripheral blood reveals a severe leukopenia within 1 or 2 days and this is progressive, the counts generally falling below 1,000 cells at the end of 1 week. However, red blood cells and platelets are maintained at or near the normal levels for about 1 week. During the

first day after injection cellular necrosis also takes place in the gastro-intestinal tract and testes.

The latent phase is followed by a period lasting for a few days or about 1 week, characterized by progressive apathy, anorexia, weakness, weight loss, diarrhea, and finally extreme prostration and death. During this time aplasia of bone marrow and atrophy of lymphoid organs persist, the gastro-intestinal damage is largely repaired, while the injury to the testes is progressive. Leukocytes of the blood continue to fall, reaching levels of a few hundred cells shortly before death. Moderate anemia also develops. Platelets are markedly diminished after the first week and this is coincident with the appearance of hemorrhagic lesions.

Death generally occurs from 9 to 18 days after radioactive material is administered. At autopsy significant lesions are confined to the hemopoietic system, intestinal tract, and testes. In some animals, despite severe injury and depletion, the lymph nodes and spleen show beginning or even moderately advanced regeneration. Only minimal or slight repair is observed in the bone marrow. Usually the intestinal lesions are almost completely healed at the time of death, while those of the testes reveal no significant repair. With only a few exceptions there is evidence of bacterial invasion in the form of numerous and sometimes massive necrotic bacterial foci, especially in lymph nodes and spleen (Figs. 45 to 48).

Mechanism of Radiation Death

The mechanism of radiation death has not been established. With massive doses death occurs within a few days and is associated with a shock-like state.[17] This has been attributed to a general toxemia resulting from the products of tissue destruction. Circulatory failure is present [18] and may be related to the appearance in the blood of excess histamine.[19] With smaller doses, resulting in death within a few weeks rather than days, factors in addition to toxemia assume importance, especially in combination. These include tissue anoxia,[20] hemorrhage, and bacterial invasion. Chrom [21] and Lawrence and Tennant [22] emphasized the frequency in death from experimental radiation of infection by the colon-aerogènes group whose probable portal of entry is the intestinal tract. Bacteremia was practically constant, while, in contrast, animals which died of radiation within a few days had negative cultures of blood and viscera. Our studies of rats killed by P^{32} suggest strongly that bacterial toxemia may play a rôle in causing death.

Investigations dealing with protective mechanisms may help in elucidating the mode of radiation death. Pretreatment with estrogens has been

shown to improve survival of mice after lethal x-irradiation.[23] Protection also is afforded by lead shielding of the spleen.[24] Cysteine administered to rats prior to x-irradiation in the lethal range greatly diminishes toxicity.[25] Cysteine or an intermediate appears to protect certain critical cellular constituents against oxidation.

Development of Malignant Tumors in Rats Injected with P^{32}

One of the late effects of radioactive phosphorus poisoning was the development of malignant neoplasms.[26] These consisted of osteogenic sarcomas and squamous cell carcinomas involving the face. The former were associated with widespread atypical proliferative lesions of bone resulting from radiation injury.

Summary

Lethal doses of radioactive phosphorus produced extensive morphologic lesions in rats. These were similar to previously described effects of radiation, from either external or internal source, in both animals and man.[8,15,17,20,27,28] Similar changes result also from the nitrogen mustards.[29,30] By virtue of its distribution, P^{32} acted as a powerful hemopoietic poison producing aplasia of the bone marrow and hypoplasia of the lymphoid tissues of the body, including spleen and thymus. This was reflected in the peripheral blood by rapidly developing leukopenia, thrombocytopenia, and anemia. In addition, the intestinal tract, especially the small bowel, and the gonads were sensitive to radiation. Organs such as liver, pancreas, adrenals, kidney, heart, and brain were radio-resistant.

Destruction of tissue occurred rapidly, being well developed from 6 to 12 hours after injection of the P^{32}. The principal mode of cell destruction was by karyorrhexis. Most of the resulting débris was removed by the second or third day. Involution of the hemopoietic organs was marked at the end of 24 hours and was then progressive over the next few to several days.

During the period of necrosis and involution the animals usually were in good condition and asymptomatic. However, this latent interval was followed by progressive inanition, debility, and weight loss, terminating in death within 3 weeks after administration of the radioactive material. At the time of death there were aplasia of bone marrow, atrophy of lymphoid tissues, and persistent leukopenia, but the hemopoietic system often revealed beginning or even fairly substantial regeneration; the intestinal damage was largely repaired while the injury to testes was progressive.

In animals which recovered there was usually good repair of bone

marrow and lymph nodes after a few months. Regeneration was less effective in spleen and thymus, and in the testis generally ranged from slight to negligible.

One of the late effects of P^{32} poisoning was the development of malignant neoplasms.

The mechanism of radiation death is not established. With massive doses of P^{32}, after which death takes place within a few days, the outcome is usually attributed to a general toxemia resulting from extensive destruction of tissue. With LD_{50} doses of P^{32}, after which death occurs within a few weeks, the almost constant presence of infectious lesions suggests that bacterial toxemia may be significant.

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[*Illustrations follow*]

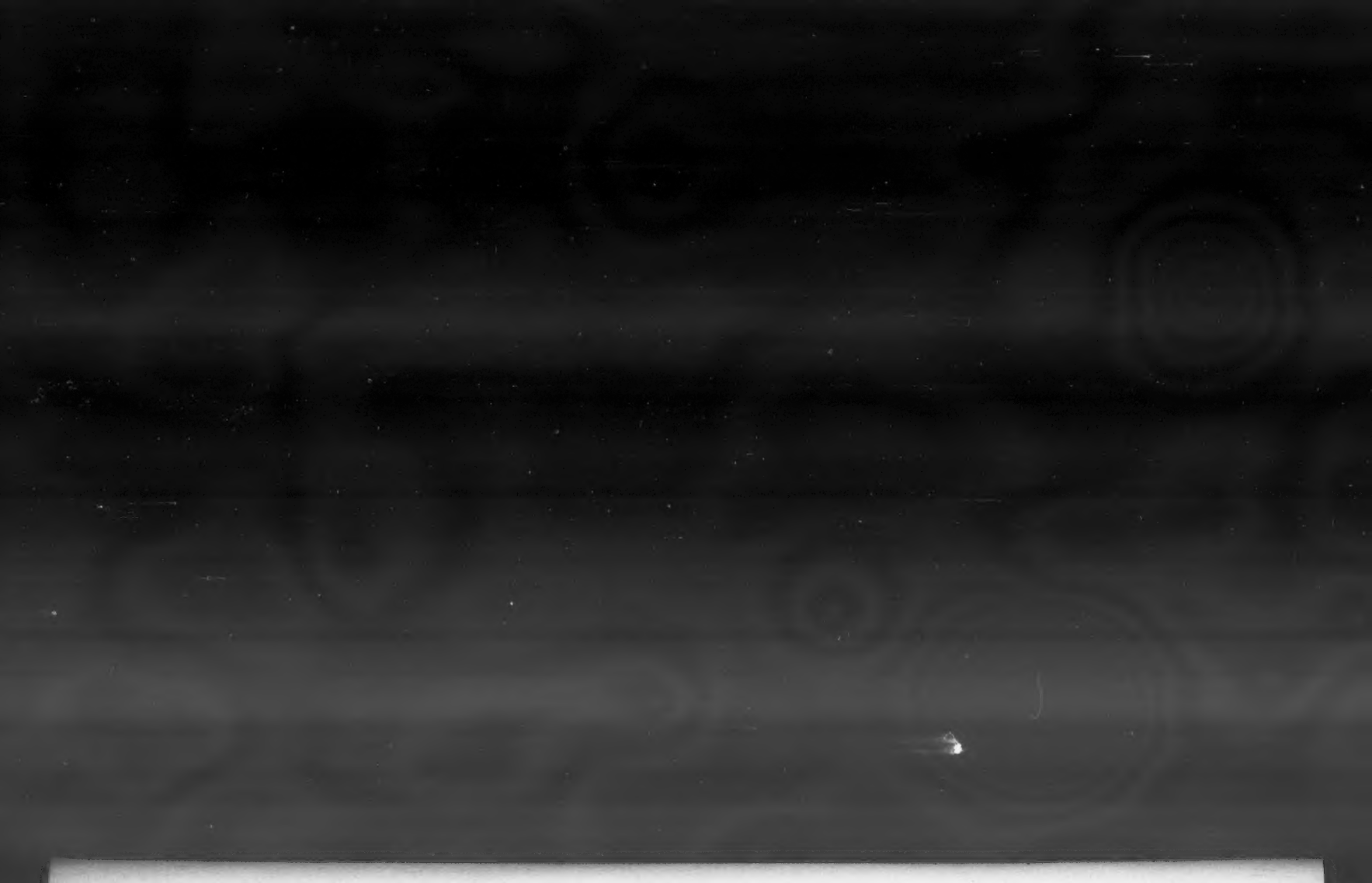
DESCRIPTION OF PLATES

PLATE 30

All microscopic sections are from rats which received an LD_{50} dose of P^{32}. Stain is hematoxylin and eosin.

FIG. 1. Radioautogram of femur from a rat sacrificed 3 days after injection of 10 μc. of P^{32} per gm. of body weight.

FIG. 2. Radioautograms of organs from a rat sacrificed 3 days after injection of an LD_{50} dose of P^{32}. The upper figure provides a key for the identification of these tissues, as follows: (1) thymus, (2) spinal cord, (3) brain, (4) liver, (5) bone marrow, (6) spleen, (7) kidney, (8) heart, (9) adrenal, (10) testis, (11) duodenum, (12) skin, (13) urinary bladder, (14) cervical lymph nodes, (15) lung, (16) skeletal muscle, (17) ileum, (18) jejunum, (19) stomach, (20) large intestine, (21) pancreas, (22) thyroid gland, trachea, and esophagus.



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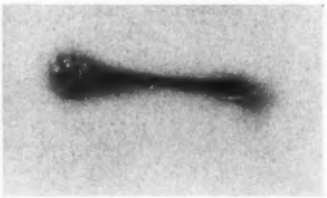

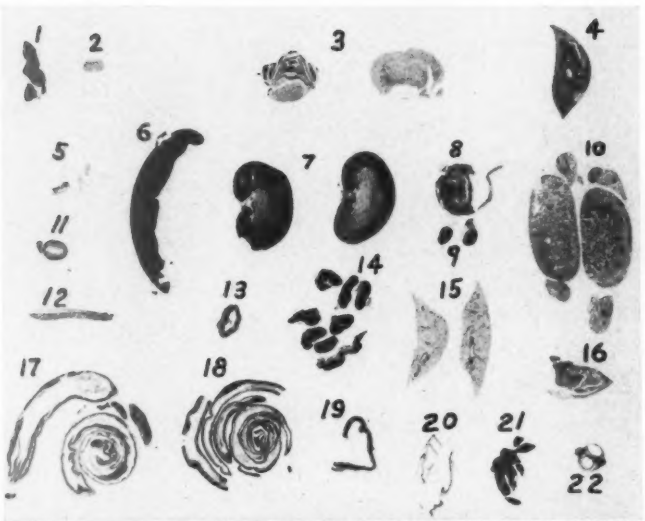

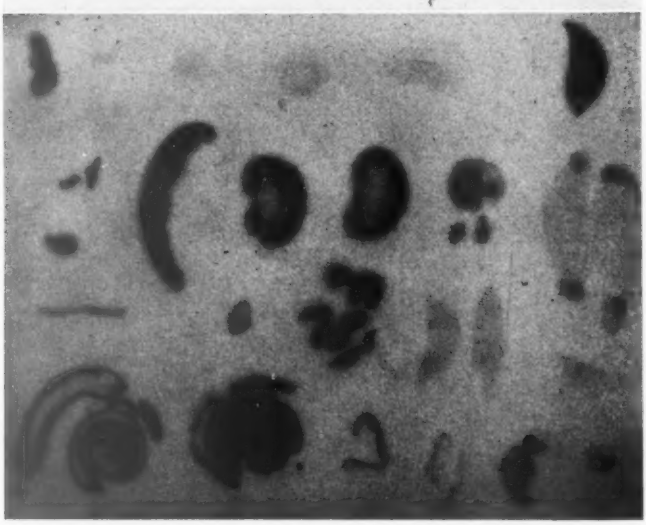

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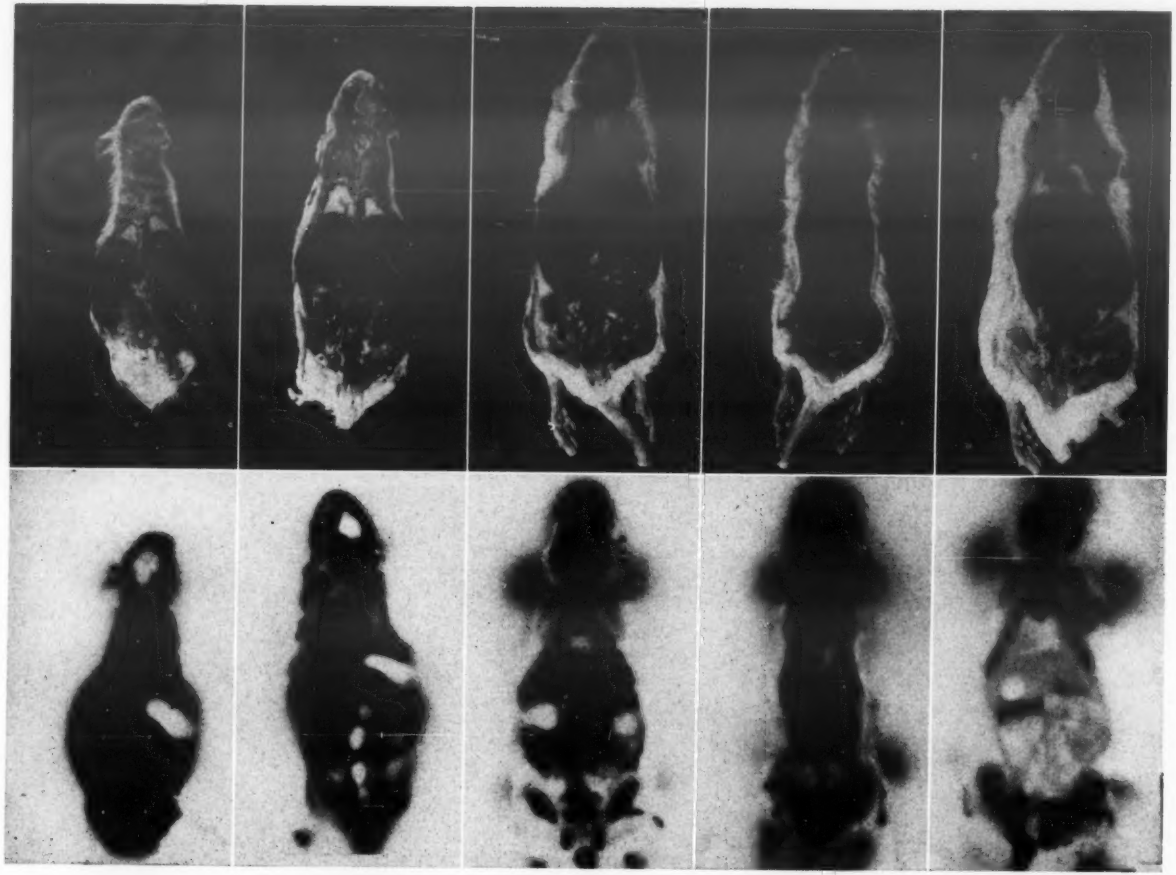
PLATE 31

FIG. 3. Whole body radioautograms made at intervals of 1 to 168 hours after injection of 200 μc. of P^{32}.



1 hr. 6 hrs. 24 hrs. 72 hrs. 168 hrs.

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Radioactive Phosphorus Poisoning in Rats

PLATE 32

FIG. 4A. Bone marrow 12 hours after injection of P^{32}. Fat cells are present and there is distinct hyperemia. × 210.

FIG. 4B. Normal marrow for comparison.

FIG. 5A. Bone marrow 24 hours after injection of P^{32}. Cells are shrunken and irregular in shape, nuclei are pyknotic and sometimes fragmented. × 460.

FIG. 5B. Normal marrow for comparison.

FIG. 6. Bone marrow 3 days after injection of P^{32}. Severe cellular depletion, hyperemia, and fatty infiltration are present. × 165.

FIG. 7. Bone marrow 10 days after injection of P^{32}, showing marked aplasia. × 160.

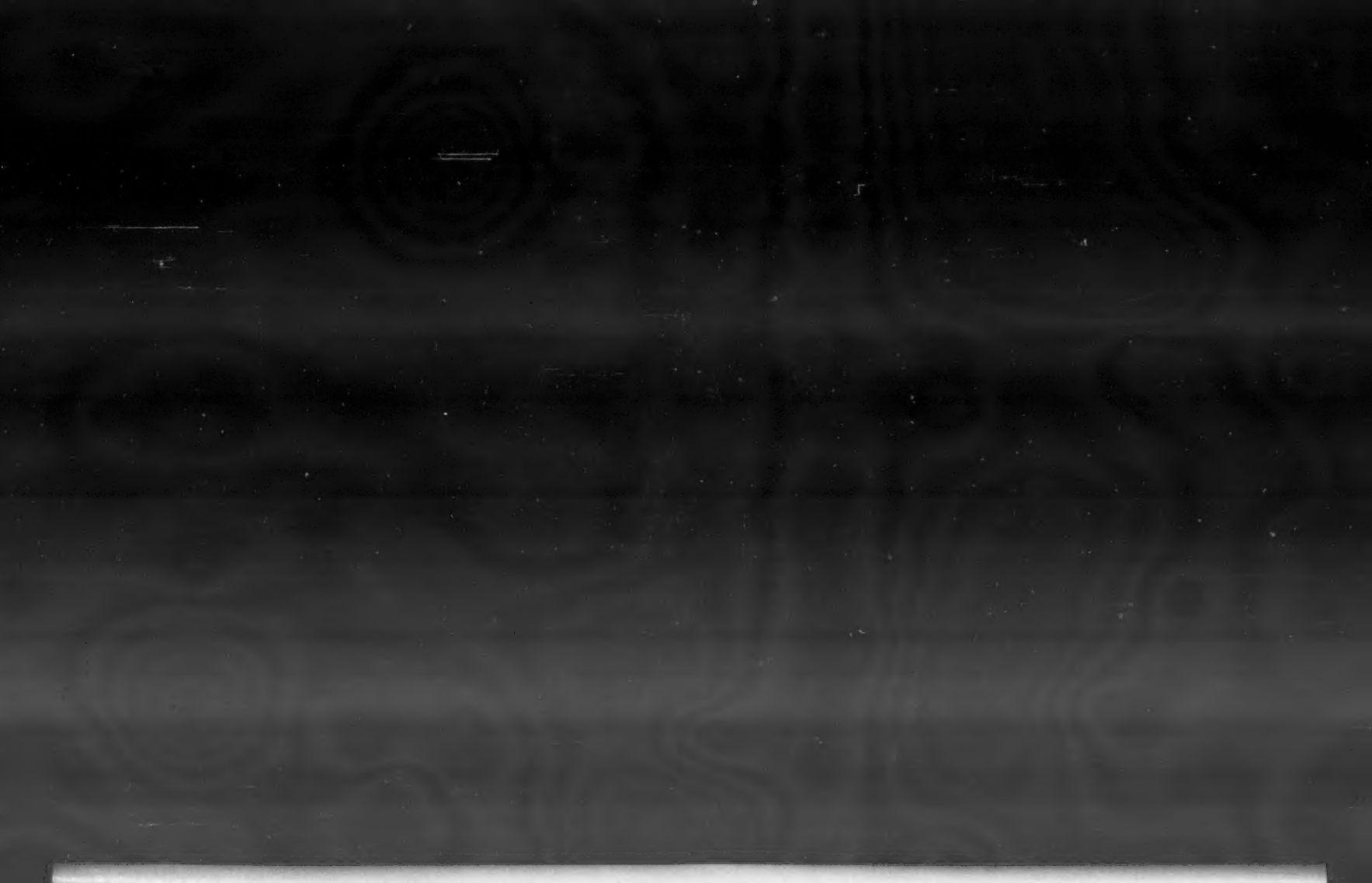
FIG. 8. Bone marrow 10 days after injection of P^{32}. Focus of necrosis with karyorrhexis. × 680.

FIG. 9. Bone marrow 10 days after injection of P^{32}. Nuclear pyknosis and rhexis. × 680.

FIG. 10. Bone marrow 12 days after injection of P^{32}. Early regeneration, principally myeloid, with mitotic activity. × 415.

FIG. 11. Bone marrow 3 weeks after injection of P^{32}. Replacement of fat by expanding groups of cells. × 173.

FIG. 12. Bone marrow 3 months after injection of P^{32}. Marrow repopulated with hemopoietic cells of all types. × 220.



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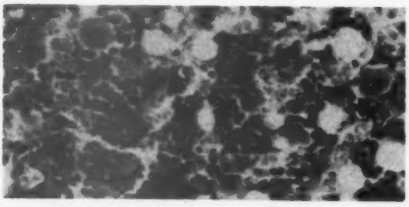

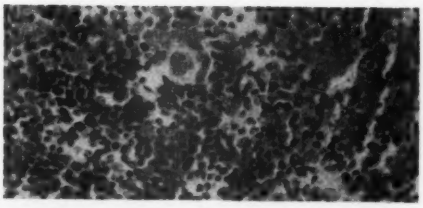

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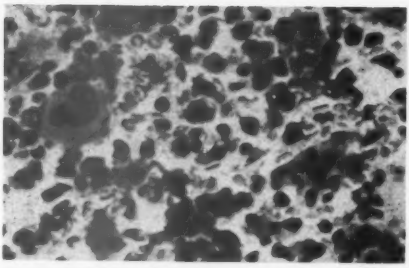

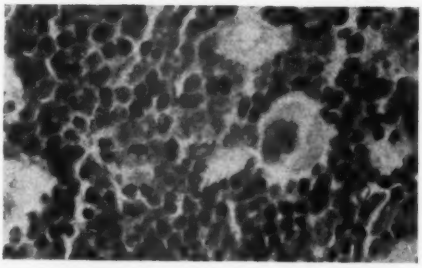

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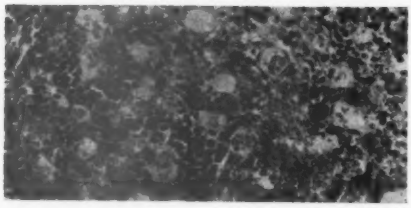

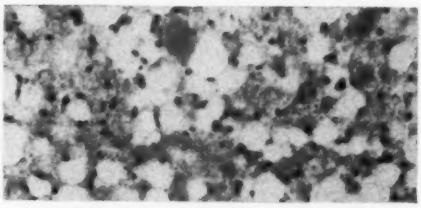

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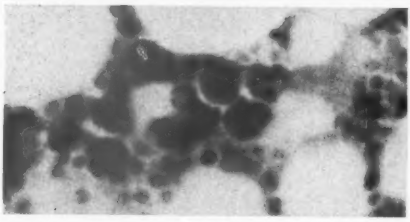

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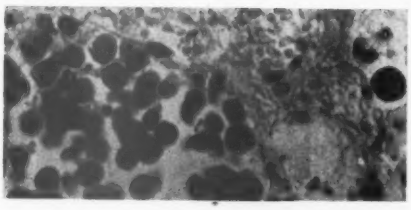

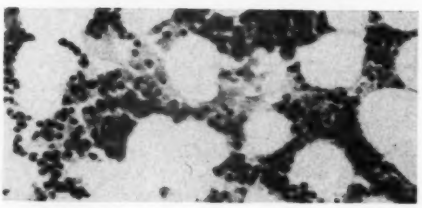

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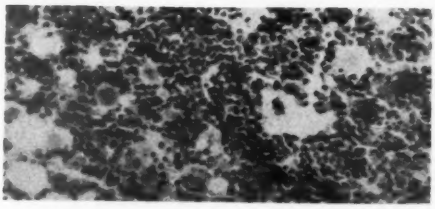

PLATE 33

FIG. 13. Lymph node 6 hours after injection of P^{32}. Pyknosis, deformity, and fragmentation of lymphocytes. Phagocytosis of nuclear particles. × 415.

FIG. 14. Lymph node 12 hours after injection of P^{32}. Cortical necrosis of lymphocytes with extensive karyorrhexis. × 430.

FIG. 15. Lymph node 10 days after injection of P^{32}. Marked pyknosis and deformity of nuclei. × 680.

FIG. 16. Lymph node 10 days after injection of P^{32}. Karyorrhexis of lymphocytes with extracellular dispersion of chromatin particles. × 680.

FIG. 17. Lymph node 5 days after injection of P^{32}. Prominence of stroma and vascular bed following depletion of cells in cortex and medulla. × 109.

FIG. 18. Lymph node 10 days after injection of P^{32}. Early focus of regeneration in cortex. × 210.

FIG. 19. Lymph node 2 weeks after injection of P^{32}. Groups of proliferating lymphocytes in medulla. Cortex still depleted and inactive. × 165.

FIG. 20. Lymph node 3 weeks after injection of P^{32}. Advanced repair in cortex with large germinal centers in follicles. × 102.

FIG. 21. Spleen 12 hours after injection of P^{32}. Cellular necrosis within germinal center of follicle. Pyknosis and rhexis of nuclei with numerous phagocytized particles. × 400.

FIG. 22. Spleen 24 hours after injection of P^{32}. Extensive depletion of white pulp. Disappearance of germinal centers. × 126.

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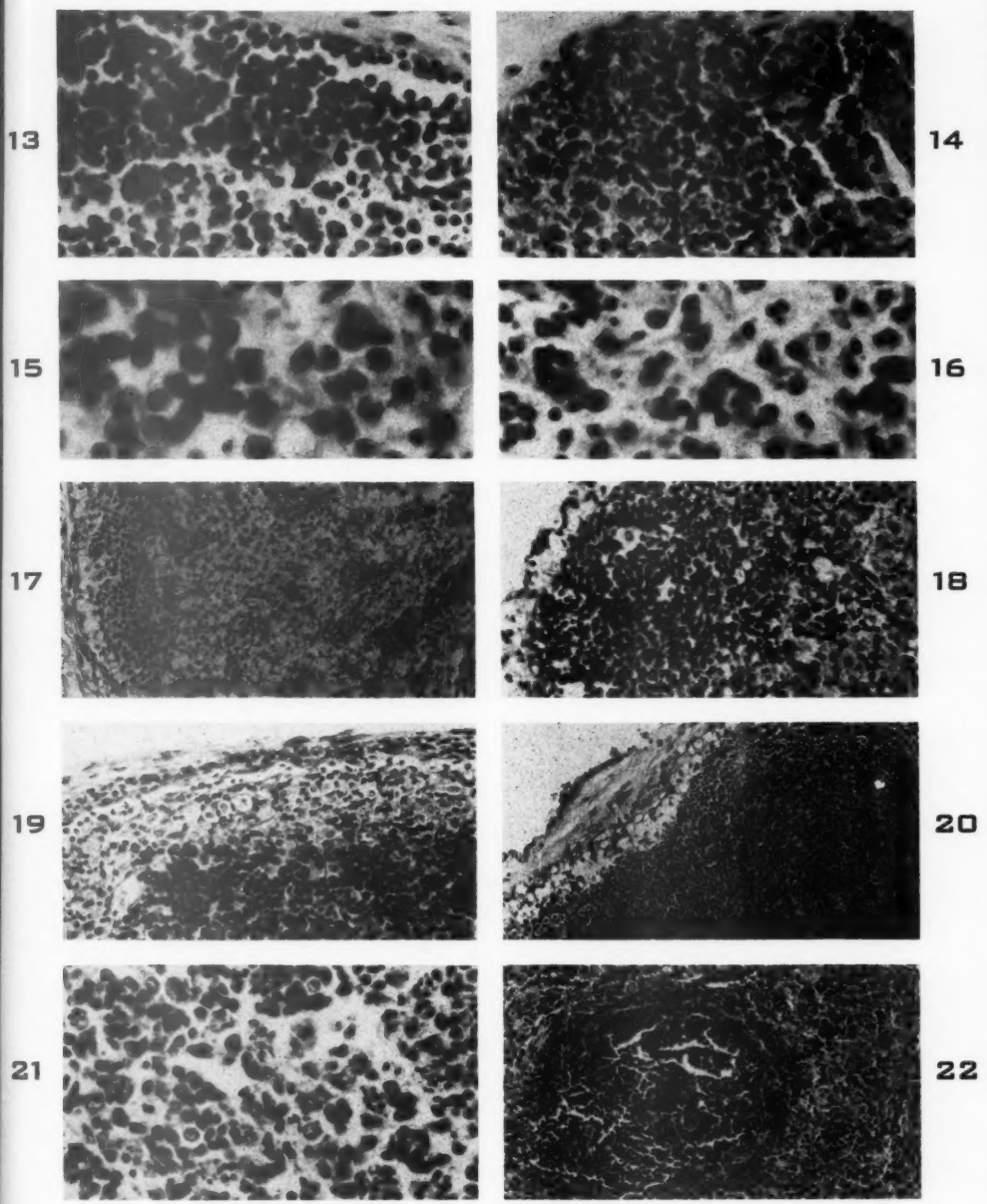

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PLATE 34

FIG. 23. Spleen 11 days after injection of P^{32}. Pyknosis and deformity of lymphocytes. × 680.

FIG. 24. Spleen 10 days after injection of P^{32}. Advanced cellular depletion resulting in prominence of stroma. Necrotic bacterial lesion in center. × 160.

FIG. 25. Spleen 11 days after injection of P^{32}. Large phagocytes containing hemosiderin pigment. × 680.

FIG. 26. Spleen 10 days after injection of P^{32}. Regeneration in red pulp while follicle in center remains inactive. × 173.

FIG. 27. Spleen 2 weeks after injection of P^{32}. Advanced repair in red pulp involving erythroid, myeloid, and lymphocytic elements. Follicle still inactive. × 142.

FIG. 28. Spleen 2 months after injection of P^{32}. Reconstitution of follicle with lag in red pulp. × 142.

FIG. 29. Thymus 3 days after injection of P^{32}. Marked involution of both cortex and medulla. × 142.

FIG. 30. Thymus 11 days after injection of P^{32}. Advanced involution with condensed connective tissue stroma. × 160.

FIG. 31. Thymus 10 days after injection of P^{32}. Pyknosis, deformity, and rhexis of lymphocytes. × 680.

FIG. 32. Thymus 3 days after injection of P^{32}. Early repopulation of medulla with lymphocytes. × 142.

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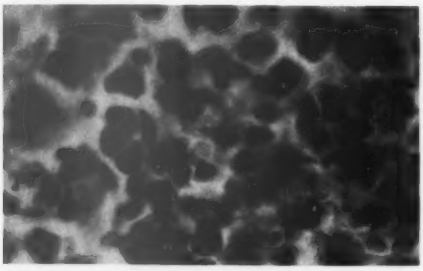
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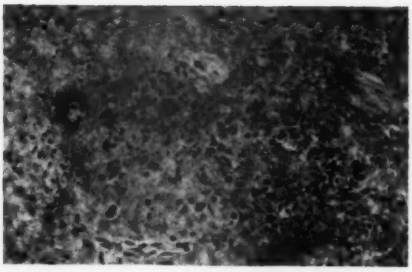
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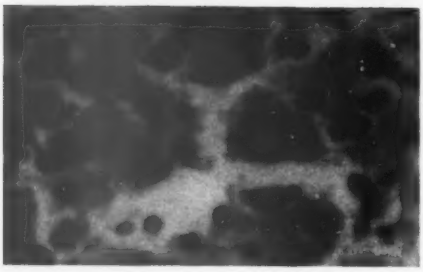
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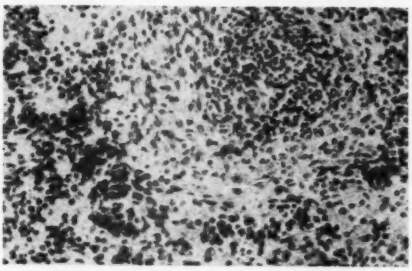
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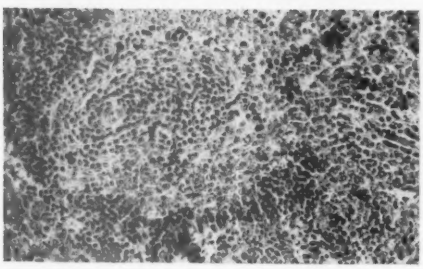
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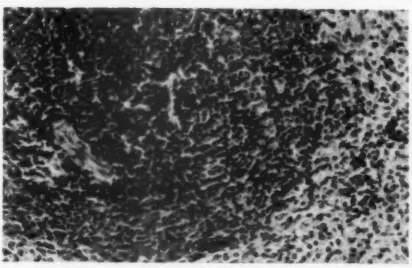
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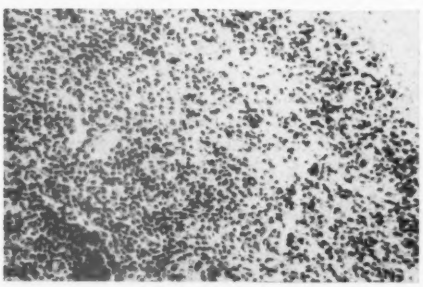
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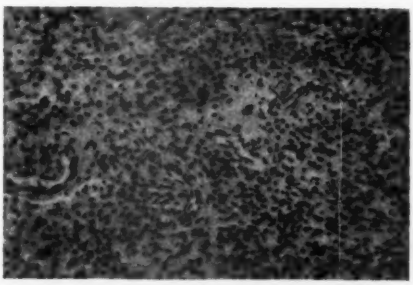
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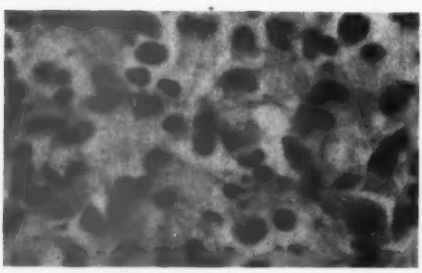
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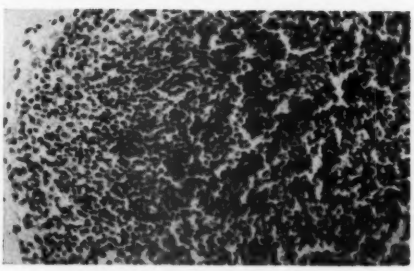
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PLATE 35

FIG. 33. Thymus 10 days after injection of P^{32}. Progressive regeneration. Dense masses of lymphocytes in cortex. × 142.

FIG. 34. Thymus 3 months after injection of P^{32}. Regeneration is virtually complete. × 158.

FIG. 35. Small intestine 6 hours after injection of P^{32}. Base of crypts shows epithelial necrosis. There is rhexis of nuclei with dispersion of chromatin particles. × 342.

FIG. 36. Small intestine 5 days after injection of P^{32}. Complete repair of damaged epithelium. × 268.

FIG. 37. Testis 24 hours after injection of P^{32}. Loss of orderly arrangement and dislocation of cells into lumen. × 154.

FIG. 38. Testis 3 weeks after injection of P^{32}. Although spermatogenesis is present, there is marked depletion of spermatogonia. × 228.

FIG. 39. Testis 3 months after injection of P^{32}. Tubular atrophy is advanced. × 184.

FIG. 40. Testis 4 months after injection of P^{32}. Complete tubular atrophy. × 154.

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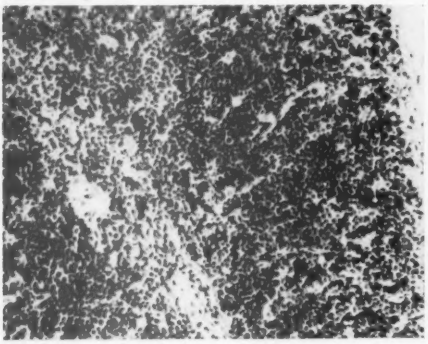

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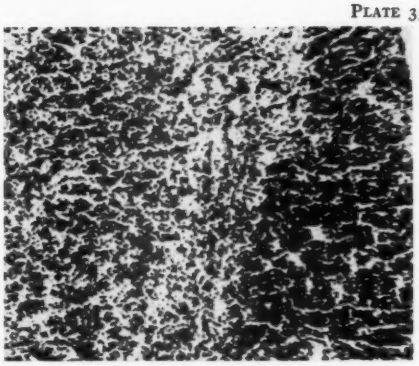

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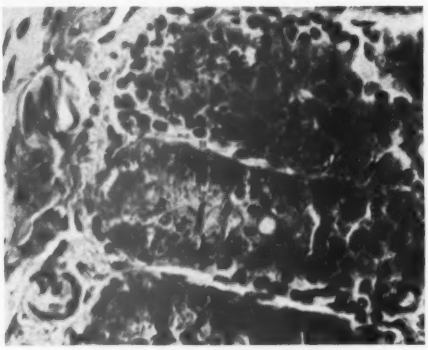

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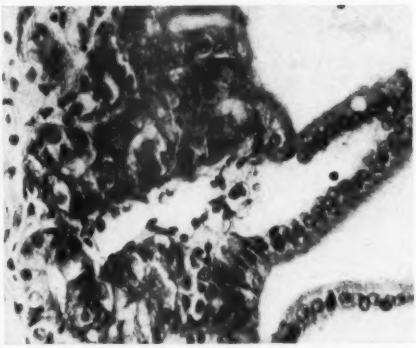

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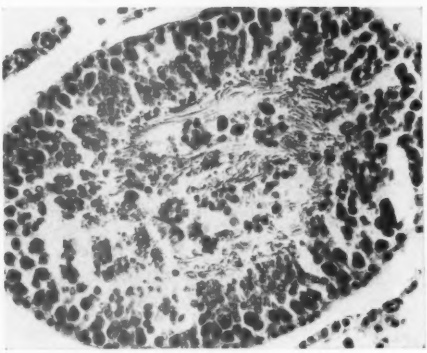

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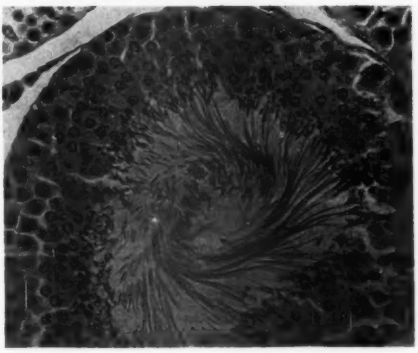

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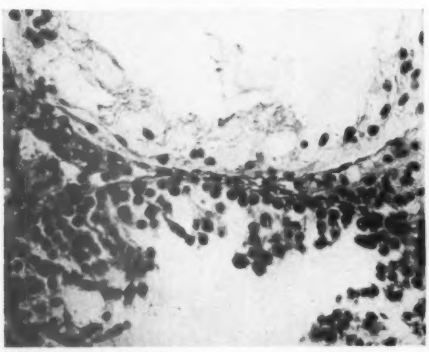

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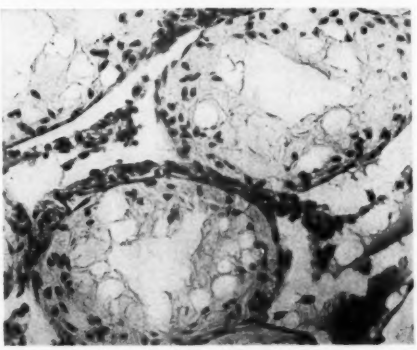

PLATE 36

FIG. 41. Ovary 14 days after injection of P^{32}. Necrosis of follicular cells. Atypical coarse chromatin in young ovum. × 200.

FIG. 42. Ovary 8 months after injection of P^{32}. Marked atrophy of organ with prominent interstitial stroma. × 102.

FIG. 43. Ovary 8 months after injection of P^{32}. Collections of lutein-like cells in stroma. × 200.

FIG. 44. Ovary 8 months after injection of P^{32}. Degenerative change in primary and young ova. × 200.

FIG. 45. Lymph node 11 days after injection of an LD_{50} dose of P^{32}. Large masses of bacteria within cortex. × 79.

FIG. 46. Adrenal 11 days after injection of an LD_{50} dose of P^{32}. Necrotic lesion containing bacteria. × 160.

FIG. 47. Cecum 14 days after injection of P^{32}. Extensive necrosis and ulceration of mucosa with many bacterial masses. × 102.

FIG. 48. Trachea 13 days after injection of P^{32}. Ulcerative and necrotizing lesion of inner lining with massive bacterial invasion. × 102.

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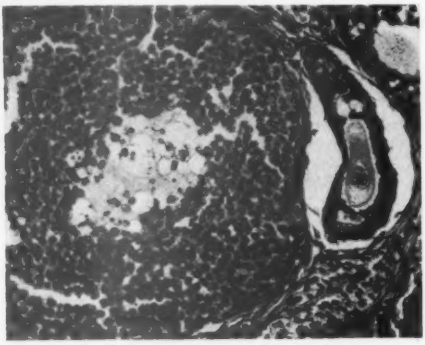

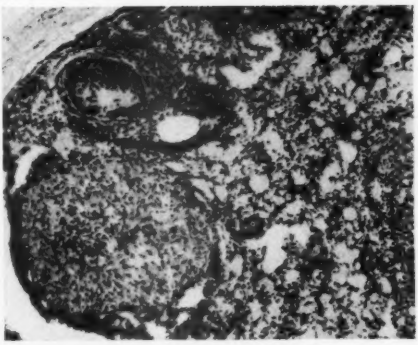

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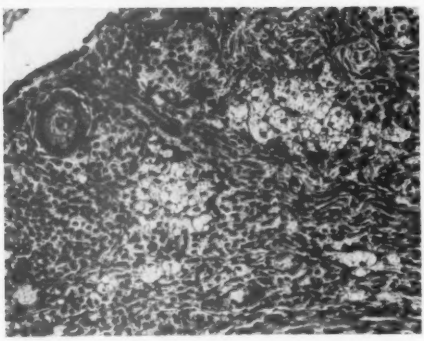

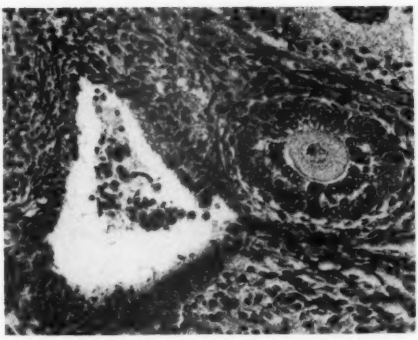

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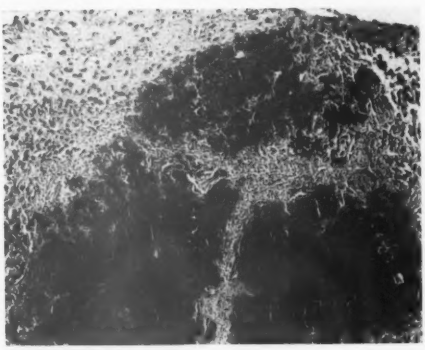

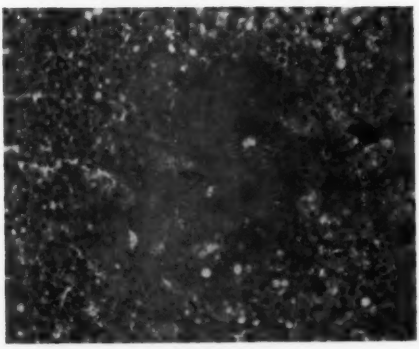

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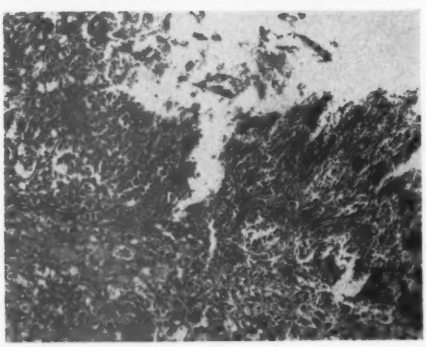

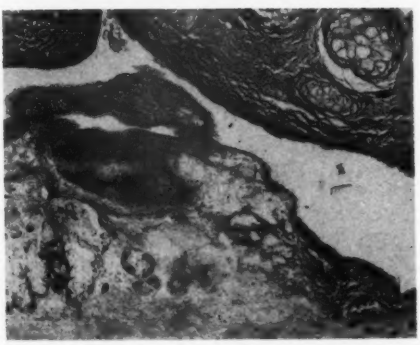

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Koletsky and Christie

Radioactive Phosphorus Poisoning in Rats

CHANGES IN THE ENDOCARDIUM OF PIGS SIMULATING THE RHEUMATIC STIGMATA OF MAN *

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During the last few years it has become the clinical custom to diagnose an obvious mitral lesion with a presystolic murmur not simply as old endocarditis with stenosis of the mitral orifice, but as rheumatic mitral stenosis. This is true particularly if the history discloses what is accepted nowadays as evidence of rheumatic disease; not a clear-cut history of polyarthritis, fever, etc., but a recollection by either the patient or the parent, who, having first denied any previous diseases, when confronted with pertinent questions, admits that there was in childhood a rare nosebleed or growing pains. Nowadays such unreliable symptoms are often taken as anamnestic evidence of rheumatic fever.

If such patients die and necropsies are performed, old endocarditis with or without stenosis of the mitral orifice is usually confirmed. Pathologists, too, have adopted the habit of specifying the type of endocarditis and usually call this old rheumatic endocarditis. The diagnosis of "rheumatic" in such instances is not based upon the only indisputable evidence of rheumatic fever—the demonstration of Aschoff bodies—but is influenced in part by the clinical terminology and in part by certain anatomical criteria which are usually designated as rheumatic stigmata. These rheumatic stigmata involve changes in the pericardium, the left atrium, and the valves, principally the mitral valve. It is usually said that pericardial adhesions—circumscribed obliteration of the pericardial sac near the apex—are probably of rheumatic origin. Certain thickenings of the mural endocardium of the atrium with rugae-like elevations, often situated on the posterior wall of the left atrium just above the posterior leaflet of the mitral valve and arranged in parallel lines, also are often considered characteristic. The mitral valve discloses fibrous thickenings at the line of closure and sometimes also thickenings of the entire leaflet with vascularization. Also a number of microscopic changes, particularly the presence of mononuclear cells arranged in a palisading fashion perpendicular to the free margin of valves with newly formed blood vessels, and perivascular fibrosis in the myocardium, are often thought to be evidence of an old rheumatic in-

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fection. The question immediately arises whether such old lesions are characteristic enough to warrant the specific diagnosis of old rheumatic heart disease, or whether the old endocarditis might be bacterial in origin. In the healed stage there may be little difference between old rheumatic endocarditis and old bacterial endocarditis.

It was thought that should there be a species of animals not subject to rheumatic fever but which does contract bacterial endocarditis, examination of the heart valves would be valuable in disclosing the presence or absence of healed endocarditis. If healed endocarditis were found, it would be of interest to compare the changes with those usually called rheumatic stigmata. It was soon learned that the common pig occasionally has acute endocarditis. Such lesions occur in swine erysipelas and also in hog cholera.[1,2] While such acute endocarditis is often discussed in veterinary literature, it was of interest to learn that little mention is made of studies on deformed heart valves in pigs, although such changes often have been noted. This is understandable since veterinary investigations usually are stimulated by economic and practical factors, and, as a result, descriptions of old endocarditis or old heart lesions are only rarely encountered in veterinary literature.[3]

Erysipelothrix rhusiopathiae, the causative agent of swine erysipelas, is the most frequent cause of endocarditis in pigs.[1,4] Four different clinical forms of this disease are recognized in the pig [4]; an acute septic form, a more chronic form almost always causing a vegetative endocarditis (the affected animals often die of this condition), an urticarious form occurring with or without associated internal lesions, and an arthritic form, occurring in older animals. It should be noted here that swine erysipelas occurs also in sheep in which a polyarthritis often is observed. Endocarditis occurs also in hog cholera,[5,6] a disease which is caused by a virus but which is often complicated by *Salmonella suipestifer* or, rarely, by swine plague.[5,6] Occasionally, also, endocarditis caused by a streptococcus [1] has been reported in the swine. Careful examination of the veterinary literature, with particular reference to diseases occurring in pigs, discloses nowhere the mention of rheumatic fever or rheumatic endocarditis, or a disease resembling it. Nowhere did we find any mention of Aschoff nodules, or of structures resembling them, in the myocardium of pigs.

This study is based on a gross examination of 1,000 hearts of pigs and on a macroscopic and microscopic examination of 850 additional porcine hearts. These hearts were examined at the stockyards in Chicago * at the time of evisceration, about ½ hour after death. Two hun-

* We are greatly indebted to Dr. R. L. Digman, Mr. A. W. Macker, and Dr. E. E. McDaniels of Swift & Company, Chicago, for this material.

dred pigs of the first group of 1,000 were less than 8 months of age, and the rest were 1 to 7 years of age, the average age being about 4 years. It must be emphasized that these pigs were sent to the stockyards for the sole purpose of processing for the food market. None of them had appeared diseased. The hearts were removed from the remainder of the organs and then examined in the usual manner. Specimens were taken for microscopic studies from any area disclosing gross abnormal changes in 850 hearts. In those hearts in which the gross findings were characteristic of old endocarditis, sections were taken from several areas of the endocardium, myocardium, and pericardium. Because of the large amount of material, special attention in this study could be paid only to the mitral valve and left atrium, where most of the changes were noted. Also, because of the nature of the material, practically no bacteriologic examination could be carried out.

Results

Tables I, II, and III give a short summary of the pertinent findings in these porcine hearts. Of 1,000 hearts studied grossly, 200, as mentioned previously, were from pigs less than 8 months of age. In none of these 200 hearts was there any evidence of endocarditis. The hearts were seemingly normal in size, and the endocardium throughout was

Table I

Summary of Gross Findings in 1000 Porcine Hearts

Involved region	Number of hearts with endocarditis	
	Recent	Old
Mitral valve	14	93
Aortic valve	8*	11
Left atrial endocardium	14*	97†
Left ventricular endocardium	3*	23

* These hearts showed also a recent endocarditis of the mitral valve.
† The mitral valve was involved in 93 of these hearts.

smooth, glistening, and transparent. Also the pericardium was normal. However, in the older group definite gross changes were encountered in the endocardium in approximately 10 per cent of the hearts examined. In 20 instances of the combined group definite recent endocarditis was found. In 12 of these endocarditis was recognized by the succulent appearance of the cusps or leaflets, recent ulceration, and hemorrhage. In 8 instances definite vegetative endocarditis was present. The lesions were practically similar in all. They were found on both the aortic and mitral valves. The leaflets of the mitral valve were thickened, whitish, and often retracted. The chordae tendineae were shortened and some of them were fused. The cusps of the aortic valve were somewhat re-

tracted and grossly thickened. Huge cauliflower-like, pale yellow vegetations sprinkled with red were present on both valves. The superficial portions of the vegetations occasionally were calcified. The mural endocardium of the left atrium and ventricle was thickened and wrinkled and a few small hemorrhages were scattered throughout these wrinkled areas. The myocardium contained grossly several scattered, whitish

TABLE II

Summary of Gross and Microscopic Findings in the Endocardium of 850 Porcine Hearts

Involved region	Number of hearts with endocarditis	
	Recent	Old
Mitral valve	6*	82
Aortic valve	2†	6‡
Left atrial endocardium	5†	78‡
Left ventricular endocardium	2†	16‡

* Only 2 of these hearts were examined microscopically.
† These hearts also showed a recent endocarditis of the mitral valve.
‡ The mitral valve also was involved in these hearts.

areas of fibrosis and a few larger scars replacing heart muscle fibers. In 2 of these instances, the valve and vegetations were examined microscopically. The vegetations consisted principally of fibrin, many polymorphonuclear leukocytes, a few round cells, and endothelial leukocytes; but also many fibroblasts and connective tissue fibers were found throughout the vegetations, principally at their bases. Many blood

TABLE III

Summary of Microscopic Findings in the Myocardium of 850 Porcine Hearts

Type of lesion	Number of hearts
Acute myocarditis	3
Subacute myocarditis	14
Chronic myocarditis	2
Myocardial fibrosis	38
Myocardial infarction	5

vessels were seen in these regions. Nowhere could there be noted a characteristic arrangement of the inflammatory cells. Clumps of bacteria were encountered often and large amorphous masses of calcium could be recognized in the more superficial portions of the vegetations. The endocardium was covered by small strands of fibrin in which there were foci of young granulation tissue. Beneath the endocardium a new formation of connective tissue, endothelial leukocytes, a few polymorphonuclear leukocytes, and lymphocytes were found. Occasionally, minute areas of necrosis were present in the midst of the cellular aggregates. The myocardium contained small and larger infarcts in the stage of organization and many areas of perivascular fibrosis. Several small

arteries within the myocardium were occluded by emboli. Bacteriologic studies could be conducted in only 2 instances. In both of these, *Erysipelothrix rhusiopathiae* was found. In 2 instances a grossly recognizable, acute mural endocarditis was encountered, involving only the endocardium of the left ventricle. Most of the inflammatory cells in these lesions were polymorphonuclear leukocytes.

The mitral valve showed evidence of old endocarditis in 82 of the 850 hearts. The endocardium of both leaflets was thickened. The leaflets were often retracted and almost invariably fused, causing either a moderate stenosis of the mitral orifice, or insufficiency of the mitral valve, or both. The thickening involved not only the line of closure of the valve, but sometimes the entire free margins. The chordae tendineae were invariably involved. They were thickened, two or three of them were fused, and often they were shortened also. Changes in the mural endocardium, or rather subendocardium, of the left atrium were striking. Sometimes only small, insignificant, whitish patches of fibrosis were present, covered by endocardium. Often large areas of fibrosis were found just above the mitral valve, consisting mainly of white rugae or folds of subendocardial fibrosis in parallel arrangements. Sometimes these rugae again showed several minute indentations. Occasionally, plaques of fibrosis were undermined, forming pocket-like structures, the openings of these pockets being directed towards the mitral valve. Most commonly the patches of fibrosis were found on the posterior surface of the left atrium at varying distances from the mitral valve. Only occasionally an entire segment of the atrial endocardium contained grayish white striae.

The mural endocardium of the left ventricle was involved in an old process in 16 of the 850 hearts. White, opaque, thickened areas were present here and there, but were more frequent on the endocardial surface of the interventricular septum. Occasionally, some of these circumscribed thickenings also were undermined, causing the formation of pockets, their openings being directed toward the aortic valve. Small areas of hemorrhage occasionally were encountered in both the mural and valvular endocardium.

The pericardium of only a few of the hearts was involved. Grossly, there were circumscribed adhesions between the visceral and parietal pericardium in a few white, thickened patches on the visceral pericardium. Microscopically, there were some thickening of the pericardium, increased vascularity, and a moderate round cell infiltration. On the whole, pericardial involvement was very slight.

The outstanding microscopic changes in the mitral valves of these 82

hearts were an increase in connective tissue, with or without hyalinization, and marked vascularization. Some of the newly formed vessels had thick walls due to increase in the muscularis and multiplicity of elastic lamellae. Sometimes their lumina were eccentric. Areas of perivascular infiltrations of lymphocytes and a moderate, more or less diffuse, infiltration of lymphocytes and endothelial leukocytes were noted also. Here and there proliferation of lining endothelial cells of blood vessels was observed also. Areas of fibrinoid degeneration sometimes were seen. Young connective tissue with relatively many nuclei was arranged in whorls, the nuclei often assuming a parallel arrangement. Anitschkow cells were noted occasionally between other inflammatory cells. Frequently endothelial leukocytes were encountered just beneath the endocardium, being somewhat more numerous in these areas than elsewhere. Endothelial leukocytes and fibroblasts showed a tendency toward an arrangement perpendicular to the endocardium; but nowhere did the cellular arrangements and stromal changes suggest a similarity to the changes usually encountered in acute rheumatic valvulitis. There was a multiplication of the lining endocardial cells, which sometimes were three or four rows thick. In 6 of these 82 instances which grossly showed no evidence of acute vegetative endocarditis, there was also a moderate polymorphonuclear leukocytic infiltration, the cells being arranged in small groups just beneath the endothelial lining of the valve. Also, the lymphocytic infiltration was more severe in these instances. The aortic valves disclosed marked subendocardial fibrosis and new formation of blood vessels, but only a slight infiltration of lymphocytes and a few fibroblastic cells.

In 78 instances the thickened mural endocardium of the left atrium was examined microscopically. It often disclosed multiplication of lining cells and small or larger accumulations of lymphocytes and endothelial leukocytes. Few newly formed blood vessels were encountered in these areas. A new formation of connective tissue with relatively few nuclei was present beneath the endocardium with occasional fibroblasts and a moderate lymphocytic infiltration. A few cells of the Anitschkow type also were seen. Large monocytes often were found arranged in parallel rows, their long axis roughly parallel to the endocardium and only sometimes perpendicular to the endocardial surface. Just beneath the endocardium there was a reduplication and thickening of the elastic lamellae. They were arranged in parallel rows. There was a moderate increase in the lining endothelial cells and slight new formation of connective tissue between the lining cells and the elastic tissue in these regions. In the more advanced instances the elastic lamellae were more

numerous but seemed thinner than normal. They often were spread by young connective tissue having many lymphocytes and fibroblasts. Between the lining endothelial cells and the elastic lamellae in these areas there was much newly formed connective tissue with a number of new thin-walled blood vessels. In still older instances the elastic lamellae seemed to have been broken up and the inflammatory reaction to be more severe. Still later, calcification of broken up elastic lamellae was noted. Often such calcific lamellae were clumped and were surrounded by endothelial leukocytes and occasional giant cells of the foreign body type. In 5 instances more recent changes were seen, such as small accumulations of polymorphonuclear leukocytes with various areas of hemorrhages. Rarely, scattered multinucleated cells were seen in these regions, seemingly consisting of fused endothelial leukocytes. They did not resemble the multinucleated cells of the Aschoff body.

Within the myocardium, infiltrations of lymphocytes and a few polymorphonuclear leukocytes with varying amounts of fibrosis were encountered in 14 instances. Myocardial fibrosis alone, principally perivascular in type, was found in 38 hearts. A combination of both lymphocytic infiltrations and fibrosis of the myocardium, classified as chronic myocarditis, was encountered twice. Small, typical, organizing and old infarcts with connective tissue fibers and cells, and a number of endothelial leukocytes containing blood pigment granules, were found 5 times. Two of these 5 were associated with vegetative endocarditis. In both of these cases small emboli were encountered in branches of the coronary artery.

Discussion

Among 1,850 porcine hearts of all ages, or, in particular, among 1,650 hearts of apparently healthy pigs which were over 8 months old when they passed through the stockyards, an old healed valvular endocarditis was found 175 times. Most commonly the mitral valve was involved. Also old subendocardial thickenings, sometimes with the formation of pockets, were found in the mural endocardium, particularly that of the posterior wall of the left atrium. In only 4 instances was the left atrial endocardium involved in the absence of endocarditis of the mitral valve. The myocardium showed old fibrous lesions, more pronounced in perivascular areas. Some of these hearts also disclosed old circumscribed pericarditis.

A number of similar changes often are encountered in human hearts and are sometimes grouped among the so-called rheumatic stigmata. Thus, VonGlahn [7] believed that in the endocardium of the auricle, lesions

exist which are both grossly and histologically distinctly rheumatic. These lesions have been described as confined to an area just above the posterior leaflet of the mitral valve. The endocardium is made irregular by low ridges or hillocks often crowded together to form a plaque 2 cm. or more in extent. When closely aggregated the surface has a furrowed appearance, some of the furrows frequently running roughly perpendicularly to the ring of the valve. Histologically, there are collections of large cells generally forming a palisade along a band of hyaline material. Also round cells, plasma cells, and a varying number of polymorphonuclear leukocytes may be found elsewhere in the endocardium. Particular stress is laid on the arrangement of the nuclei of large mononuclear wandering cells perpendicular to the endocardial surface. The elastic lamellae are widely separated and pushed apart by various cellular accumulations.

Gross [8] also described microscopic lesions of the left auricle as they occurred in 87 rheumatic hearts. In 67 of these the disease was active and there were Aschoff bodies in the myocardium. However, 20 showed only chronic valvular disease thought to be of the rheumatic variety, with no demonstrable Aschoff bodies. In 80 per cent of the whole group microscopic lesions were found in the left auricle. These lesions were present on the posterior wall of the left atrium, at times spreading over almost the entire endocardial surface, and about the orifices of the pulmonary veins. Also irregular, low ridges and hillocks separated by furrows were described.

Hall and Anderson [9] discussed the incidence of rheumatic stigmata in hearts which are usually considered to be non-rheumatic. Of 112 non-rheumatic hearts, thickening of the leaflets of the mitral valve was found to be moderately severe in 65 instances and severe in 9. Minimal thickening of the leaflets of the mitral valve was observed in 74 instances. They believed that these lesions signified rheumatic infection.

The literature and pertinent data have been well reviewed recently by Karsner and Koletsky [10] in their study of "Calcific Disease of the Aortic Valve." Lesions possibly the result of old rheumatic involvement were noted particularly in the left atrial endocardium. They are described as not sharply defined, sometimes circular in outline, measuring 2 to 3 cm. in diameter. The surface of such lesions may present many irregularly arranged nodular ridges and projections of variable size, separated by more or less sharp furrows. While Karsner and Koletsky believed that rheumatic endocarditis of the left atrium usually occurs in hearts with deformity of the mitral valve and with more or less widely distributed microscopic rheumatic stigmata, especially of the valves, they stressed that atrial stigmata may aid in only occasional instances in

establishing rheumatic fever as a cause of equivocal valvular disease. Among 100 instances of chronic rheumatic heart disease, rheumatic stigmata were found in the left atrium 60 times. Aschoff nodules, which they considered to be the only lesion pathognomonic of rheumatic fever, were encountered only 26 times. They described the subendocardial plaques in the atrium as papillary masses, essentially fibrous, formed by proliferation of connective tissue in the innermost part of the endocardium. These authors also differentiated between the plaque of the papillary type and the slightly elevated or flat hyaline plaque, of more or less uniform width, which is found in many normal hearts, owing to loss of superficial elastica.

Rheumatic stigmata in the mitral valve usually are described as due to fibrosis, especially marked at the line of closure and often fusing it with the free border. In more deforming examples, the leaflets may be attached to one another and the attachment of the chordae tendineae may be obscured but the chordae themselves are thickened. Vascularization often is noted grossly. Adhesions of chordae and retractions, however, are infrequent. Endocardial reduplication, cellular exudation, and vascularization, especially by thick-walled musculo-elastic arteries and arterioles, are microscopic stigmata of chronic or healed rheumatic endocarditis.

Because of such and similar findings, the view usually is prevalent that rheumatic fever is a disease which is much more common than would be supposed by reference only to deforming valvular disease. A comparison of the gross and microscopic findings in these porcine hearts, particularly the changes found in the left atrial endocardium, with those constituting rheumatic stigmata in human hearts demonstrates that they are similar in many respects. Only the marked multiplication of the elastic lamellae, their eventual destruction, calcification, and the formation of elastic granulomas seem not to be present in the atrial plaques of human hearts. Koletsky [11] mentioned the presence of elastic tissue in 12 of 23 subendothelial plaques of 100 rheumatic hearts. Distortion and interruption of the elastica were observed by him in 4 non-rheumatic and 15 rheumatic hearts. He stated that, in general, in any variety of rheumatic plaque, elastic tissue may be absent or present in slight to considerable amount. Also, Gross [8] described condensation of elastic fibers in the subendocardium of the auricle and elastic distortion with patchy arrangement of elastic fibers.

With the possible exception of the behavior of the subendocardial elastic fibers, the cellular changes, musculo-elastic blood vessels, and fibrosis and hyalinization in pigs' hearts are quite similar to those interpreted as rheumatic stigmata in human heart. Whether these changes

in the elastic apparatus are characteristic of the type of reaction in this animal species, or whether they indicate a reaction to a specific causative agent cannot be ascertained.

Microscopically, neither Aschoff bodies nor any other lesions which could be explained as possible healed Aschoff bodies could be found in the myocardium of these pigs. If these pigs had rheumatic fever, then the changes in the hearts could be attributed to an old rheumatic infection. However, as mentioned before, as far as we are aware, there are no observations on record of rheumatic fever in pigs and no statement could be found as to the occurrence of Aschoff bodies in pigs' hearts. Because of the very nature of this study, no attention could be paid to possible joint involvement. However, it is known that a number of pigs with swine erysipelas develop a chronic, seemingly non-specific arthritis, although a true rheumatic arthritis has never been found in pigs. If, in the future, it can be shown that acute rheumatic fever is not a specific disease brought about by a specific organism, but the result of a hyperergic reaction toward a non-specific etiologic factor, perhaps those organisms which cause acute endocarditis in pigs may also evoke the hyperergic reaction which, perhaps, is rheumatic fever. However, if this were so, one should expect to find Aschoff bodies in the myocardium at least occasionally. Whatever it may be, this study indicates that bacterial endocarditis in pigs sometimes heals with resultant valvular endocardial and myocardial changes of which some are quite similar to those called rheumatic stigmata. Although severe stenosis of the mitral valve was not found in these hearts, it is evident from the gross findings that some degree of stenosis of the mitral orifice or insufficiency of the mitral valve was present in many of them. From this it seems clear that in man, also, every example of old endocarditis of the mitral valve and of certain old changes in the left atrial endocardium and myocardium is not necessarily the result of rheumatic fever, but may very well be the result of non-specific endocarditis brought about by various organisms. Acute endocarditis and endocarditis lenta may heal as a result of modern medical treatment. However, even before the era of modern treatment, it was noted that occasionally endocarditis lenta may heal spontaneously.[12] Since it is obvious that these pigs must have had acute bacterial endocarditis which had healed, it is conceivable that also in man, acute bacterial endocarditis may heal more commonly than is usually accepted, even without treatment. The end stages of such endocarditis in man, just as in these pigs, may resemble end stages of rheumatic endocarditis and simulate the so-called rheumatic stigmata. From this it seems clear that every presystolic murmer over the apex of the

heart must not be interpreted as stenosis of the mitral orifice on a rheumatic basis and be attributed to old rheumatic endocarditis of the mitral valve. Also, every example of old endocarditis of the mitral valve seen at necropsy, with or without deformities of the orifice and with various areas of thickening in the atrial endocardium or other evidence of so-called rheumatic stigmata, should not be classified immediately as old rheumatic endocarditis.

Summary

This study is based on the gross examination of 1,000 hearts of the common pig and on the combined gross and microscopic examination of 850 additional pigs' hearts. Two hundred of the first group were less than 8 months of age. None of these 200 hearts showed gross evidence of heart disease. Evidence of old healed endocarditis was found in approximately 10 per cent of the remaining 1,650 hearts. A recent endocarditis was found in 20 hearts, and in 8 it was characterized by huge vegetations. In 2 of the latter, *Erysipelothrix rhusiopathiae* was recovered from the vegetations. Among the 850 hearts examined grossly and microscopically, old healed endocarditis of the mitral valve was found in 82 instances: of the left atrial mural endocardium in 78 instances; of the aortic valve in 6 instances; and of the left ventricular mural endocardium in 16 instances. The myocardium was involved in 62 instances: by acute myocarditis in 3; subacute in 14; and true chronic myocarditis in 2 instances. Myocardial fibrosis was found 38 times and myocardial infarction 5 times. Lesions which could have been interpreted as either recent or old Aschoff bodies were never found. Although chronic arthritis is not rare in the pig, true rheumatic fever or a rheumatic arthritis has not been observed. It seems likely that the old valvular endocarditis and the old lesions of the left atrial endocardium were the result of healed bacterial endocarditis, caused by organisms relatively commonly encountered in acute endocarditis in pigs. A marked resemblance was noted between the gross and microscopic changes of the mitral valve and of the left atrium in these porcine hearts and the changes in human hearts usually regarded as the result of old rheumatic endocarditis. Because of this resemblance, the inference is made that many of the heart lesions in man diagnosed as old rheumatic endocarditis, in the absence of Aschoff bodies or structures interpreted as healing Aschoff bodies, may also be regarded as the results of a previous bacterial endocarditis.

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DESCRIPTION OF PLATES

PLATE 37

Lesions of the mitral valve and atrial endocardium from porcine hearts.

FIG. 1. Thickening and retraction of the mitral leaflets.

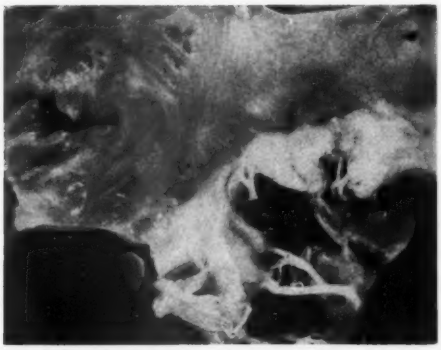
FIG. 2. Diffuse thickening of the mitral valve and the chordae tendineae.

FIG. 3. Fibrosis of the atrial endocardium with formation of pockets.

FIG. 4. Marked fibrosis of the atrial endocardium with many ridges.

FIG. 5. Fibrosis of the endocardium with bizarre pocket formation just above the posterior leaflet of the mitral valve.

FIG. 6. Old endocarditis with shrunken leaflets, marked thickening of the chordae tendineae, and localized fibrosis of atrial endocardium.



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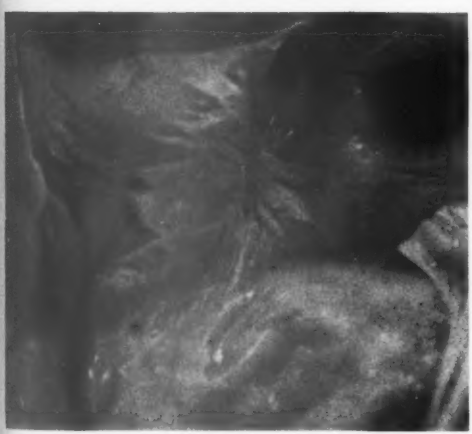

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Porcine Lesions Simulating Rheumatic Stigmata

PLATE 38

Microscopic changes of the mitral valve.

FIG. 7. Multiplication of endocardial lining cells and young fibroblasts. Iron hematoxylin and eosin stain. × 180.

FIG. 8. Many lymphocytes and endothelial leukocytes. Iron hematoxylin and eosin stain. × 180.

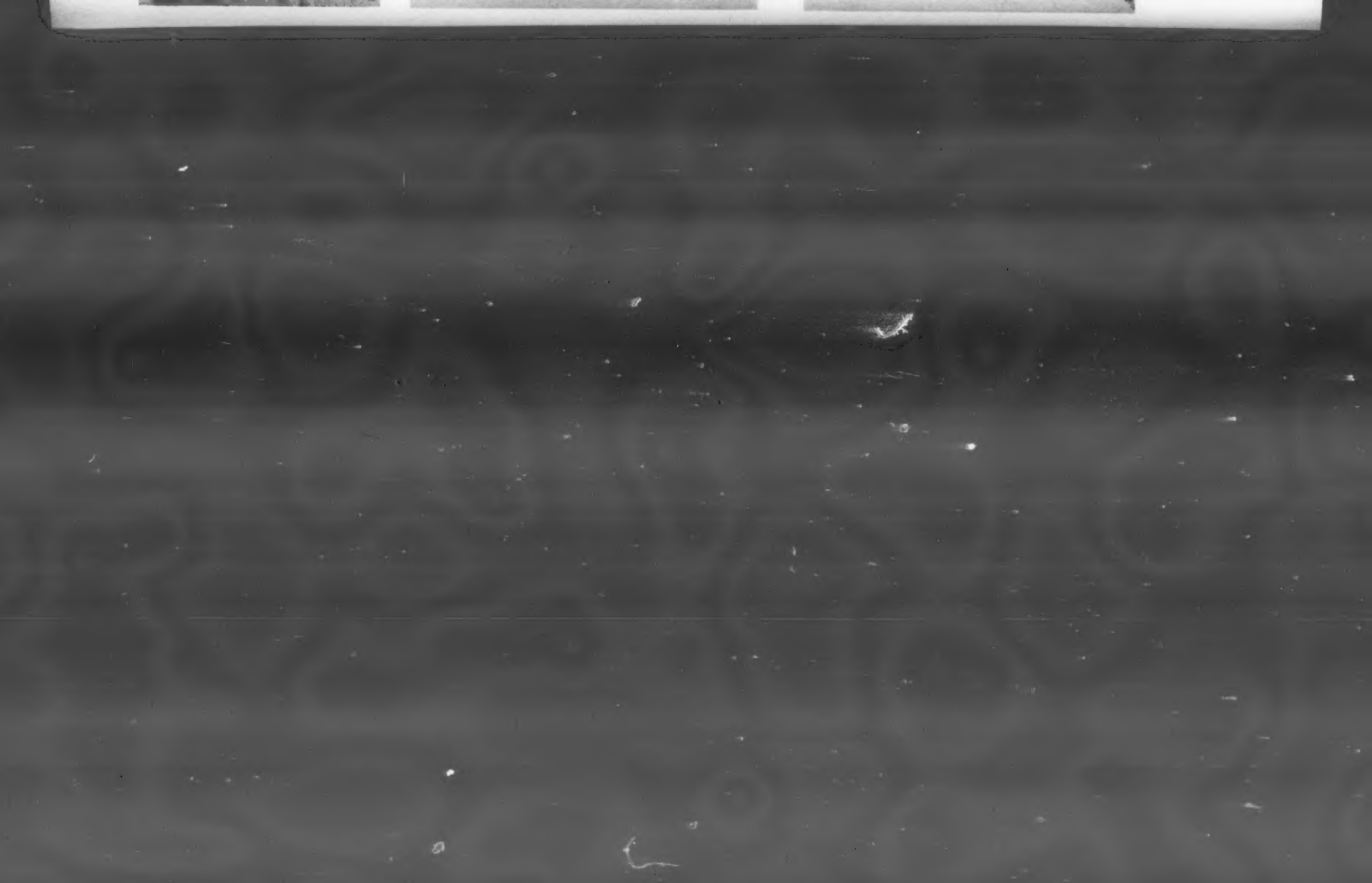
FIG. 9. Proliferation of endothelial lining cells with many lymphocytes and endothelial leukocytes. Iron hematoxylin and eosin stain. × 220.

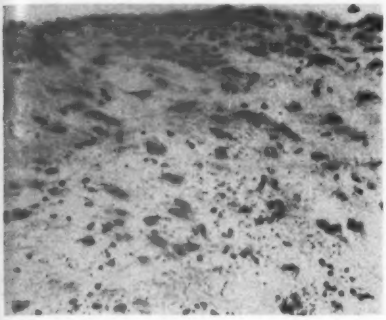
FIG. 10. Similar to Figure 9. Hematoxylin and eosin stain. × 160.

FIG. 11. New formation of connective tissue cells arranged perpendicularly to the surface. Iron hematoxylin and eosin stain. × 180.

FIG. 12. Young connective tissue fibers with a few lymphocytes. The connective tissue cells in general are arranged perpendicularly to the free surface. Iron hematoxylin and eosin stain. × 180.

FIG. 13. The walls of newly formed blood vessels are thickened. Hematoxylin and eosin stain. × 180.





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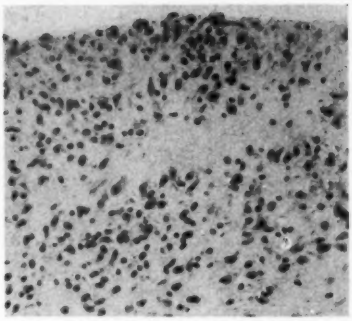

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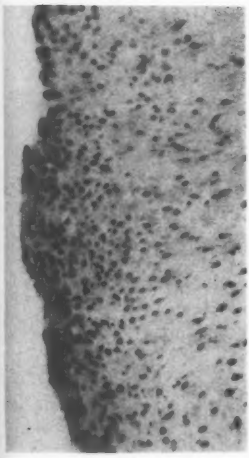

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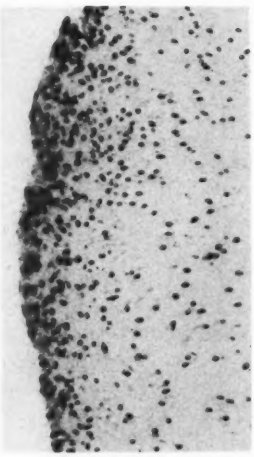

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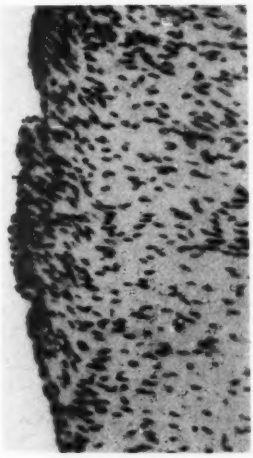

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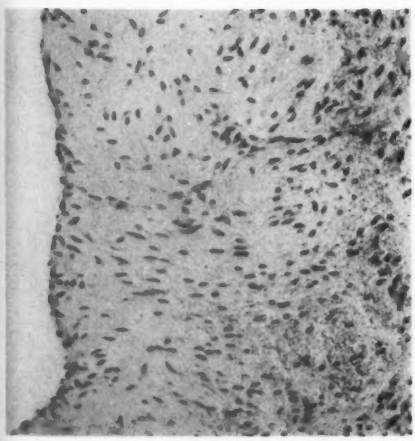

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PLATE 39

Microscopic changes of the atrial endocardium.

FIG. 14. Multiplication of the subendocardial elastic lamellae. Orcein stain. × 180.

FIG. 15. Multiplication, thinning, and splitting-up of elastic lamellae and small calcified elastic lamellae just above the center of the field. Orcein stain. × 180.

FIG. 16. Broken-up elastic lamellae with many lymphocytes and endothelial leukocytes. Also of note is a slight thickening of the endocardium. Orcein stain. × 360.

FIG. 17. Spreading of the elastic lamellae by inflammatory cells and edema. Also of note are calcific deposits and necrosis of the endocardium. Orcein stain. × 180.

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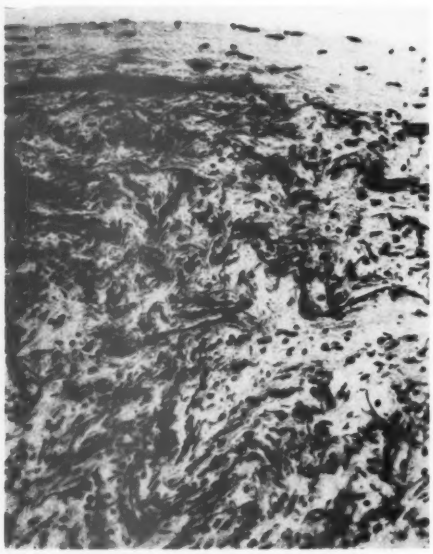

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PLATE 40

Changes in the myocardium.

FIG. 18. Subacute myocarditis, with many lymphocytes and young connective tissue cells. Iron hematoxylin and eosin stain. × 360.

FIG. 19. Perivascular fibrosis. Iron hematoxylin and eosin stain. × 180.

FIG. 20. Perivascular fibrosis with a few inflammatory cells in the adventitia. Hematoxylin and eosin stain. × 360.

FIG. 21. Peripheral portion of a small organized infarct. Of note are the newly formed blood vessels. Hematoxylin and eosin stain. × 360.

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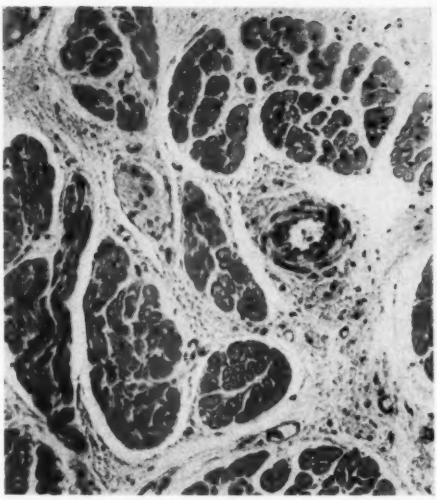

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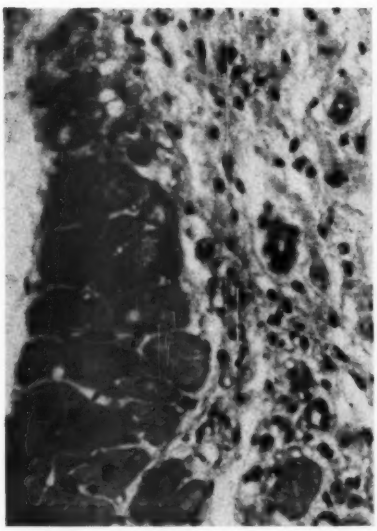

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Porcine Lesions Simulating Rheumatic Stigmata

GLANDULAR ADIPOSE TISSUE ASSOCIATED WITH CYTOTOXIC SUPRARENAL CONTRACTION AND DIABETES MELLITUS *

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Subtotal and total bilateral destruction of the adrenal glands may be accompanied by anatomical changes suggesting compensatory activity for the loss of the normal adrenal cortex. Among these changes, the formation of cortical nodules, often composed of bizarre and giant adrenal cortical cells, has been noted most frequently in cases of cytotoxic contraction.[1-9] It has been assumed that the nodules arise from cortical cells which remained after the injury to the adrenal glands as a whole. In other instances, growth of either accessory or aberrant adrenal cortical tissue has been described following destruction of the adrenals due to various causes.[1,6,10-12]

The pathologic studies leave little doubt, however, about the practical insignificance of the compensatory changes as far as maintaining life and preventing adrenal insufficiency are concerned. Yet they do present good anatomical evidence for what may happen when the normal interrelationship between the adrenal cortical hormones and the pituitary adrenocorticotrophic hormone is severely altered in man, and are of considerable interest from that point of view.

A case of bilateral adrenal destruction has been studied recently in the Department of Pathology of the Stanford University School of Medicine. In addition to areas of regeneration in the adrenal glands, there were unexpected changes in the periadrenal fatty tissue and in the fat along the spermatic vessels which will be described in the present report. Moreover, the adrenal disease developed in a diabetic child. This occurrence, in itself, was of interest and instructive.

REPORT OF CASE

The patient was a white schoolboy, 18 years old, who had been known to have diabetes since the age of 12. When first seen at the age of 16 by one of us, his diabetes had been fairly well controlled by daily doses of 30 units of crystalline insulin and 25 units of protamine insulin. The results of physical examination were normal; the blood pressure was 120/80 mm. of Hg. The blood sugar 4 hours after lunch was determined to be 182 mg. per cent. During the following 4 months, the daily dosage was increased gradually to 45 units of crystalline and 25 units of protamine zinc insulin, the average monthly blood sugar levels being 140 mg. per cent. During the following year, the patient developed mild insulin reactions, necessitating reduction to 30 units of crystalline insulin and 18 units of protamine zinc insulin daily.

* Received for publication, April 5, 1950.

Six months before the patient's death, following unusual exercise, he developed a severe insulin reaction, requiring hospitalization. He responded well to glucose therapy, but developed meningismus. Lumbar puncture revealed an initial pressure of 240 mm. of water. Routine studies of the spinal fluid, including culture, were negative. The blood sugar was 204 mg. per cent at the time of the meningismus. The patient recovered completely from this episode.

During the following 5 months, because of frequent moderately severe insulin reactions, it became necessary to decrease insulin further, so that just before his last hospital admission his daily dosage was 25 units of crystalline insulin and 12 units of protamine zinc insulin. The blood sugar at this time was 113 mg. per cent. A roentgenogram of the chest during this period showed no abnormality.

Five days before his death, the patient became unconscious and was hospitalized again. There was an acetone odor on the breath, and 2 plus acetone was found in the urine. Treatment with intravenous saline solution was started. On receipt of the values of the blood sugar and CO_2-combining power, 60 mg. per cent, and 39 volumes per cent respectively, glucose was added to the infusion, but the patient failed to respond. Routine studies of the blood and urine revealed no other abnormalities. Examination of the spinal fluid revealed an initial pressure of 460 mm. of water, a positive Pandy test, and 12 erythrocytes and 4 lymphocytes per high-power field. Cultures of the spinal fluid were negative, as were tests for virus encephalitis. During the remaining 4 days, the temperature rose gradually to 42° C. and the patient died without regaining consciousness. The blood pressure was constant at 110/80 mm. of Hg. Except during the initial hospital day, the urine remained free of acetone bodies.

During the 2-year period of observation, results of routine physical examinations every 3 months were normal; the blood pressure remained constant at 120/80 mm. of Hg.

The final clinical diagnoses were: (1) insulin reaction, severe; (2) cerebral hemorrhage, gross or microscopic, due to insulin reaction.

SUMMARY OF POST-MORTEM EXAMINATION

Necropsy was done 7 hours after death. The body measured 64 inches in length and weighed 145 lbs. External inspection showed a well developed boy with normal male distribution of hair and beginning growth of a beard. The testes were in the scrotal sac and were normal in size.

The anterior mediastinum contained a prominent thymus gland which weighed 30 gm. The heart (260 gm.) was normal. The aorta appeared hypoplastic and measured 4.0 cm. in maximum circumference in the ascending portion. The lungs were heavy (1540 gm.) and contained a large amount of edema fluid. The liver (2100 gm.) contained much blood which escaped freely from cut surfaces, but was otherwise normal. The portal system was not unusual. The spleen weighed 260 gm. and contained numerous closely spaced malpighian bodies, 1 to 2 mm. broad. The pancreas weighed 30 gm. and appeared normal. Numerous greatly enlarged lymph nodes were found throughout the abdominal cavity. Many of these had nodular surfaces which, upon sectioning, proved to be large lymphoid follicles. The kidneys and the lower uri-

nary tract were normal. The testes weighed 23 gm., and the seminal vesicles were well developed and contained mucoid material. The thyroid gland was small and weighed 10 gm. The gastro-intestinal tract was normal but contained prominent lymphoid follicles in the mucosa.

The adrenal glands were not identified at necropsy. The entire fatty tissue extending from the suprarenal region to the pelvis on each side was dissected out *in toto*, and the specimens were labelled for later identification. The strips of fatty tissue were placed in Zenker's formaldehyde solution for 20 hours, washed thoroughly in running water overnight, and chromatization was continued for 24 hours in Müller's fluid. The fat was then cut serially at 2 mm. intervals, and samples were prepared for microscopic examination. During this procedure, brown tissue was visible grossly on each side in the usual position of the adrenal glands.

The brain and meninges weighed 1700 gm. and appeared wet. There was no evidence of meningitis, and intracerebral lesions were not found. The pituitary gland weighed 324 mg., which was considered normal for the patient's age.

Summary of Microscopic Findings

Pancreas. The acinar tissue was normal in the pancreas. The islets were normal in size and number but were composed of small cells with little stainable cytoplasm. There was no evidence of fibrosis or hyaline deposits in the islets. Best's carmine stain on alcohol-fixed material from the liver and kidney failed to demonstrate abnormal glycogen deposits in these two sites.

Adrenal Glands. The right and left adrenal gland were similar. The cortex was absent in most places, and the medulla was bordered by a dense zone of collagenous fibrous tissue. The medulla was missing in several areas, and the thickness of the gland was composed of only two sheets of collagenous fibrous tissue with a central zone of dilated vascular channels surrounded by mononuclear cells. There were scattered nodules of irregular, large cortical cells often possessing giant nuclei and cytoplasmic pigment granules. The normal orientation of the cortical cells and the relationship to the capillary system were lacking (Fig. 1). Other areas were composed of cortical cells which were separated by strands of collagenous fibrous tissue through which lymphocytes and plasma cells were scattered. The inflammatory reaction was non-specific and varied in severity. No vascular lesions were found in the adrenal glands nor in the periadrenal fatty tissues which contained many fair-sized branches of the adrenal artery and vein.

Periadrenal and Paravertebral Fatty Tissue. The adipose cells of the periadrenal fatty tissue were transformed into fat cells of the embryonal type in many places (Fig. 2). Scattered through the altered fat were isolated cells and small groups of cells which possessed a finely granular cytoplasm stained deeply with acid dyes. Some of the cells contained small vacuoles, and in many, lipofuscin pigment granules were seen. The lobular pattern of the normal adipose tissue was maintained everywhere. The vascular bed, although increased over that seen in normal fatty tissue, lacked a definite orientation to the embryonal fat cells in most places.

Numerous sections prepared from the fat along the course of the spermatic vessels on both sides showed even more striking changes. In addition to the fat cell of embryonal type, large islets of protein-rich cells were found, which occupied the greater portion of individual fat lobules (Fig. 3). Some of the cells contained small single or multiple vacuoles, and brown pigment granules, free of stainable iron, were found in many of these cells. A definite vascular pattern was apparent here. The center of such an islet often was occupied by a large venous channel into which smaller blood vessels converged from the periphery (Fig. 4). Each of the protein-rich cells was thus bordered, on two sides at least, by thin-walled vessels which probably contributed to the alignment of the cells in column-like fashion. There was no capsule at the margin of the islets. Using Foot's modification of Bielschowsky's method, a delicate network of reticulum fibrils bordered each glandular fat cell. In view of the well developed vascular bed, it seemed most likely that the reticulum was derived from the vessels rather than being a product of the glandular fat cells. This was emphasized further by the fact that the reticulum was not demonstrable where normal adipose cells were seen and where the vascular network was much less developed.

Hypophysis. Well granulated acidophils were seen throughout the anterior lobe of the hypophysis. The basophils, although present in apparently normal numbers, had fewer cytoplasmic granules than usual. There were scattered small groups of large pale cells which possessed eccentrically placed nuclei. Aniline acid fuchsin-light green stain demonstrated granules which stained green in a few cells, and these were interpreted as large, poorly granulated basophils. There was no interstitial fibrosis. Sudan III stain applied to frozen sections showed only a few droplets of fat, no more than can be found in many hypophyses with routine fat stains.

Thyroid Gland. Lymphocytic infiltration in a few small patches was the only abnormality in the thyroid gland.

Testes. The seminiferous tubules were of normal width and were lined by spermatogenic cells showing all stages of maturation. The interstitial cells were normal in number and structure.

Thymus. The lobular pattern of the thymus was well preserved. The cortex and medulla were in approximately equal amounts. There was no evidence of fatty replacement.

Spleen and Lymph Nodes. Numerous hyperplastic lymphoid follicles were seen in the spleen and lymph nodes. In spite of the number and size of the follicles, reaction centers were rarely seen and most of the follicles were composed of small lymphocytes only.

Discussion

The reported cases of diabetes mellitus complicated by chronic adrenal insufficiency have been summarized recently by Crampton, Scudder, and Davis [13] and by Simpson.[14] The case reported here is an additional example and emphasizes once more the occurrence of progressive diminution in insulin requirements, and of increased insulin sensitivity with a decrease in adrenal cortical function.

As in the majority of cases of cytotoxic contraction, no lesions were found which elucidated the cause for the adrenal destruction. Tuberculosis, tumors, and vascular lesions were readily excluded. Although a history of an intercurrent disease was not obtained, it seems most likely that the time of decreased insulin requirements was related to the onset of the adrenal disease. At that time, the patient was 15 to 16 years old and within the period of adolescence.

While searching for the adrenal glands, the changes in the periadrenal fatty tissue and in the adipose tissue along the course of the spermatic vessels were found and attracted our special attention. The structural alterations of the fatty tissue appeared to be confined to these two areas. Even adipose tissue as close to the adrenals as that in the region of the tail of the pancreas was unchanged. The limitation of this type of change to the urogenital region may be important and, in this respect, the alterations differ from similar ones found in children and emaciated adults in whom embryonal or multilocular adipose tissue may be seen in various sites.[12] There was a further change in the adipose tissue beyond that usually referred to as embryonal fat, in that islets of protein-rich cells were supported by an organized vascular system. The cellular changes resembled those described as glandular fat and those which have been seen in the hibernating glands of animals during the period when activity is resumed.[15] Their functional significance is unknown. Cramer,[16] who studied the response of the glandular fat and

adrenal cortex to experimental alterations in the rat, was impressed with the similarity of the changes in these two sites and suggested that the glandular adipose tissue and the adrenal cortex are functionally related. Recently, Selye and Timiras [17] reported that the brown fat of the rat responded to acute stress-producing agents with discharge of lipids, so that the cytoplasm of the cells contained almost nothing but proteinaceous material. This similarity of response to stress in the adrenal cortex and in brown fat is noteworthy. In two articles, Wells [18] emphasized, as late as 1940, the many features of adipose tissue still to be studied.

The extensive transformation of the adipose tissue to glandular fat was not anticipated, and fresh material was not retained for chemical analyses. Yet the over-all histologic pattern, and particularly the development of a vascular system consisting of a collecting vein into which numerous smaller vessels converged, strongly suggest functional activity on the part of the glandular fat. It seems likely that the mechanism which leads to vascular growth in other organs during periods of increased cellular activity, also governs the growth of the capillary channels in the glandular adipose tissue. What the products of this cellular activity were, and whether they were important in obscuring some of the more obvious clinical signs of adrenal insufficiency, we do not know.

The histologic appearance of the islets of glandular fat resembling adrenal cortical cells ruled out the possibility that they were accessory adrenal glands. There was no capsule and no glomerular zone, and the columnar arrangement of the cells which is always seen in accessory adrenal tissue was lacking. Aberrant adrenal cortical tissue, although occurring at the sites where the islets were found, could reasonably well be excluded by the fact that all stages of transition from an adult fat cell to the protein-rich fat cell or glandular fat cell were seen.

Some thought ought to be given to the mechanism by which the glandular fat was formed. Because there is no reason to suggest a congenital anomaly of adipose tissue, such as has been presumed to be associated with certain tumors,[19] there remains the alternative that the glandular fat developed from previously normal fat cells. The presence of all transitional stages, already referred to, is highly suggestive of an active process of differentiation. If this concept is correct, two more factors must be assumed to have been instrumental. One would be a substance in the body fluid possessing the faculty of acting upon fat cells, and the second, a local factor concerned with the ability to respond to the environmental stimulus. The second factor would, of necessity, have to be within the adipose cells.

With respect to the first factor, there is good evidence from the low

level of the 17-ketosteroid excretion in patients with Addison's disease,[20] as well as from the fate of untreated cases, that the nodules of regenerated adrenal cortical cells are insignificant as far as the production of adrenal hormones is concerned. It is well known, also, that the absence or decreased function of an endocrine organ simultaneously removes the inhibitory effect of that organ upon the formation of its trophic hormone by the hypophysis. This relationship between hypophysis and peripheral target organ has been well established for the gonadal and thyroid glands.[21,22] It seems likely that a similar relationship exists between the hypophysis and the adrenal glands. Suggestive evidence has been presented indicating that, as the level of cortical hormones is decreased in the circulation, the pituitary gland discharges adrenocorticotrophic hormone.[23] This information was obtained in short term animal experiments.

After prolonged periods of adrenocortical deficiency, Taylor, Albert, and Sprague [24] recently have demonstrated adrenotrophic activity of sera which were obtained from patients with Addison's disease. No conclusive evidence of activity was found in the sera of normal subjects and of those with evidence of adrenocortical hyperfunction. These observations suggest that the anterior hypophysis continues to function in Addison's disease, although it remains for future studies to determine whether the rate of hormone production is altered.

Histologic investigations of the cellular changes in the anterior pituitary gland have shown that a decrease in the number of well granulated basophils occurs in cases of Addison's disease. Furthermore, Crooke and Russell [7] described the appearance of large pale cells in the anterior hypophysis, and they considered them to be chromophobes and abnormal basophils. The cytologic observations were confirmed by Nicholson [25] and by Laqueur and Bernstein.[26] Such groups of large pale cells may be present in small or fairly large numbers, and we have found them in 9 of 15 hypophyses of patients dying with Addison's disease. It has been possible in a few cells to stain granules with light green as the counterstain to aniline acid fuchsin. The granules stain blue with azocarmine and Mann's stain. We have considered these cells as basophils and have regarded the loss of granules as evidence of increased cellular activity.

Of particular importance are the observations which Groat [27,28] made in the ground squirrel. He described the differentiation of ovarian mesenchymal cells into adrenocortex-like cells in the adrenalectomized ground squirrel. Similar changes were seen in both males and females in other sites, although they are not specifically described. The groups of cells apparently functioned to some degree in that adrenalectomized

animals could be maintained on water without the addition of sodium chloride. They withstood prolonged periods of fasting as well as intact control animals did. The ovarian changes did not occur in the hypophysectomized animals, and Groat suggested that the differentiation of ovarian mesenchymal cells to adrenocortex-like cells was the result of adrenocorticotrophic hormone activity.

The findings of Groat demonstrate that the hypophysis is necessary for the development of compensatory changes outside the adrenals. A much larger body of experimental data is available concerning the dependency of the adrenal cortex proper upon the hypophysis. Smith [29] was the first to show that the adrenal cortex atrophies following hypophysectomy, and that this change can be prevented by injections of pituitary extracts. Since then, further work has shown that adrenal hyperplasia, such as can be observed following removal of one adrenal gland or in various types of stress, does not occur in the hypophysectomized animal. The many individual observations have been collected and reviewed in detail by Tepperman, Engel, and Long.[30] More recently, Sayers and Sayers [31] have shown that diminution in adrenal ascorbic acid, regarded as a delicate indicator of increased cortical activity, does not occur under stress following hypophysectomy. There is then good evidence that the hypophysis not only exerts a definite influence upon the normal adrenal gland but also controls, to an important degree, the growth of the adrenal gland under abnormal circumstances. It has been shown that the pituitary substance, capable of producing these effects, is the adrenocorticotrophic hormone.

It is considerably more difficult to evaluate the rôle of local factors within the adipose cells. The transformation of adult adipose cells to glandular fat has not been a regular observation when the adrenal glands are destroyed. Comparison of the present case with many others previously reported, and with 4 similar cases in the files of the Stanford Department of Pathology in which glandular fat was not found, suggests that the age of the patient at the time the adrenal disease develops may be important. It seems possible that certain regions derived from the tissues of the celomic wall may retain an ability to respond to appropriate stimuli during pre-adolescence and puberty but not in the adult.

The terminal episode in our patient, his progressive decrease in insulin requirement, the sensitivity to insulin, and the generalized lymphoid hyperplasia found at necropsy, indicate that the compensatory changes in both the fat and the adrenal glands did not replace the function of the normal cortex. Nevertheless, there was a paucity of the classical signs and symptoms of chronic adrenal insufficiency.

It seems possible, therefore, that the islets of glandular fat were functioning, and prevented some, although not all, of the cardinal features of Addison's disease. Indeed, the observations make one wonder whether comparable changes may not occur more frequently than indicated in the literature. Such findings as these, if confirmed, may help in understanding some of the cases which have been considered as early or less severe stages of the disease.

Summary

An 18-year-old boy was known to have had diabetes mellitus since the age of 12. At the age of 16, his insulin requirements progressively decreased and insulin reactions occurred. Severe reactions did not respond to intravenous glucose therapy in the usual rapid fashion. He died 2 years after the insulin requirements had begun to decline. At necropsy, extensive bilateral adrenal destruction of the type commonly seen in cytotoxic contraction of the adrenal cortex was found. Tiny nodules of regenerated adrenal cortical cells were present. The fatty tissue in the periadrenal region and along the spermatic vessels showed transformation of the adult adipose cells to glandular fat. The islets of glandular fat were supplied by a vascular system such as seen in organs with endocrine function.

The hypothesis has been advanced that the transformation of adipose cells to glandular fat was the result of an interaction between pituitary adrenocorticotrophic hormone, available in the body fluids after bilateral adrenal destruction, and the fatty tissue of the adrenogenital area. The possibility that the glandular fat was functioning to a limited degree would explain the fact that the adrenal insufficiency was less severe than that usually observed, and that many of the cardinal features were lacking.

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[*Illustrations follow*]

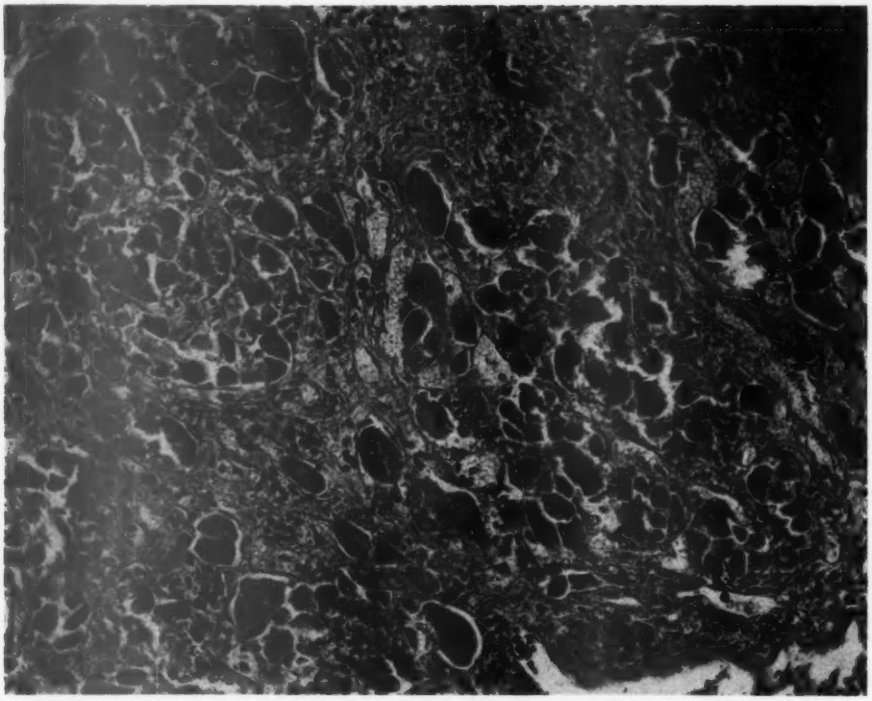
DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Nodules composed of bizarre and giant cortical cells from the right adrenal gland. × 220.

FIG. 2. Fatty tissue from the left periadrenal region showing one normal adipose cell, several embryonal or multilocular fat cells, and a few scattered glandular fat cells indicated by arrows. × 420.

1



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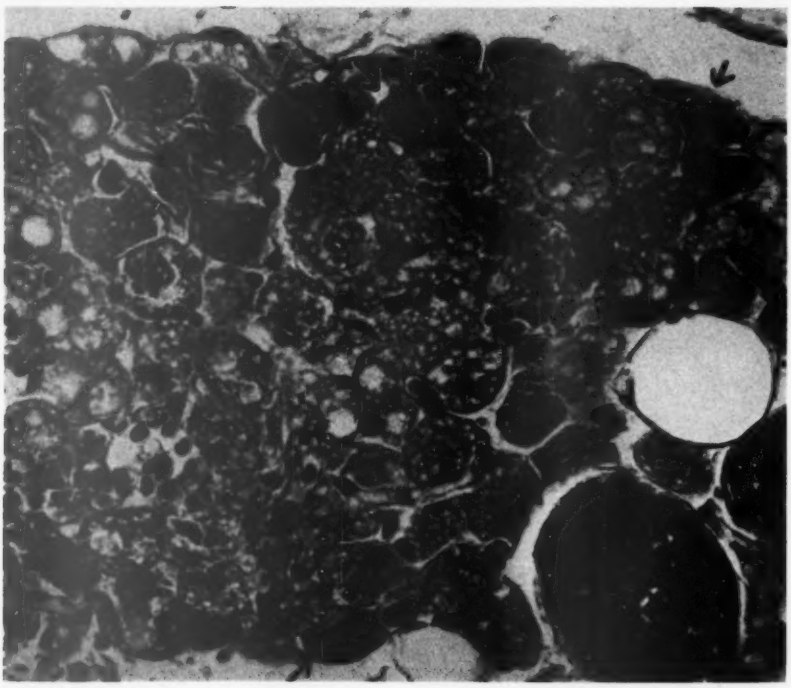


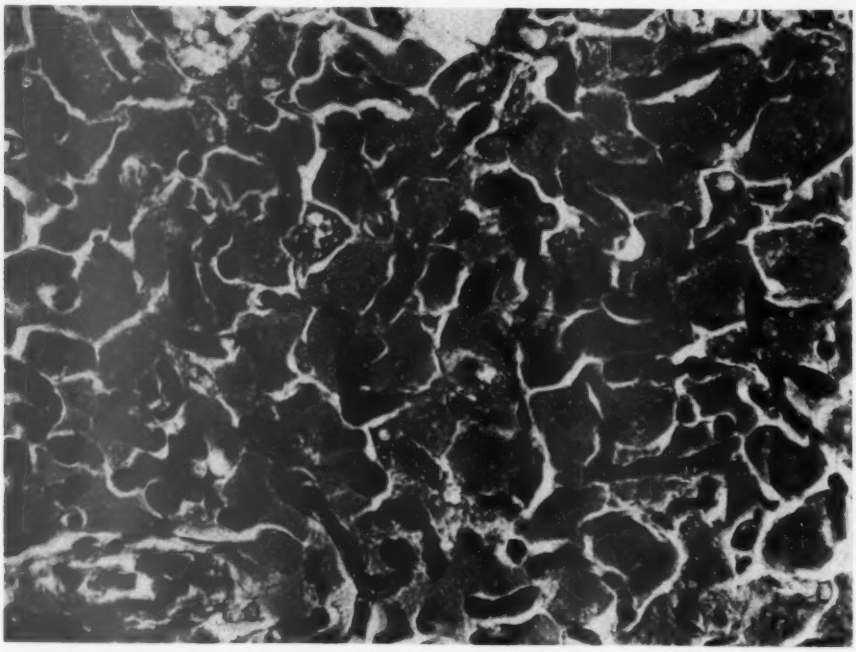
Laqueur and Harrison Periadrenal Glandular Adipose Tissue

PLATE 42

FIG. 3. High magnification of a portion of the glandular fat from the right side along spermatic vessels. Of note are pigment granules in the cytoplasm. × 420.

FIG. 4. Islet of glandular fat illustrating vascular pattern. The tissue is from an area midway between the origin of the left renal artery and the bifurcation of the aorta. × 220.

3



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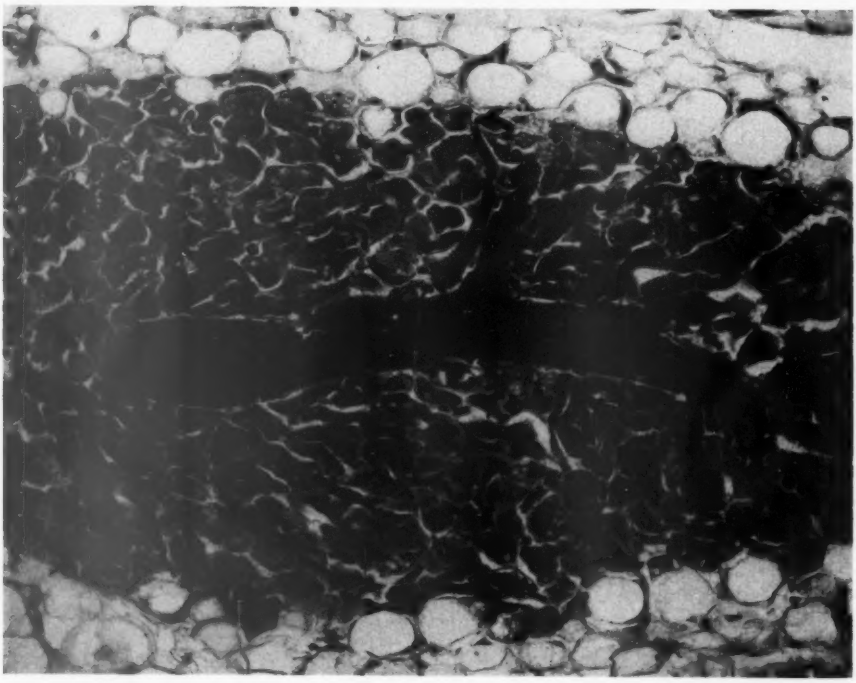

Laqueur and Harrison Periadrenal Glandular Adipose Tissue

ASYMPTOMATIC FOCAL ARTERITIS OF THE APPENDIX
EIGHTY-EIGHT CASES *

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The erroneous belief that arteritis is a rare disease should be dispelled. Eighty-eight cases of arteritis of the appendix in the modest material of a single observer can not fail to do so. An asymptomatic focal arteritic lesion of the vermiform appendix has been mentioned in demonstrations and discussions.[1-3] The first observations were made at a time when inflammation of small arteries was not foremost in the minds of pathologists. Meanwhile, arteritis has come to the fore, and the large number of cases collected justifies a comprehensive study.

The self-explanatory term focal arteritis designates single or multiple, small, circumscribed foci of necrotizing, exudative, and productive arteritis found accidentally in the vermiform appendix.

MICROSCOPIC DESCRIPTION

In cross sections of the diseased portion of an artery, the lesion appeared as a roughly circular, inflamed area with necrotic arterial wall in the center (Fig. 1). Necrosis was always found when serial sections were available. There was no thrombosis. Necrotic matter, belonging to the wall of the artery, filled the lumen in rare cases (Fig. 8). No aneurysms were ever seen, and there was no marked widening. The homogeneous, eosin-staining portions of the arterial wall gave the impression of subendothelial deposits (Fig. 5), of fibrinoid change of the media, or of necrosis; they often were a mixture of the three. Staining reactions, accordingly, were inconsistent. The homogeneous material became dark orange to brownish in the van Gieson stain, fading to a bright yellow in the course of years. It retained the blue in Weigert's fibrin stain tenaciously, and the red in Russell's stain † for hyalin as well. Metachromasia was more easily obtained with polychrome methylene blue than with methyl violet. In old foci the fibrin stain was negative, and the van Gieson stain resulted in a bright or pale yellow. Small, mostly round spaces could be seen sometimes

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† Schmorl, G. Die pathologisch-histologischen Untersuchungsmethoden. F. C. W. Vogel, Leipzig, 1928, p. 219.

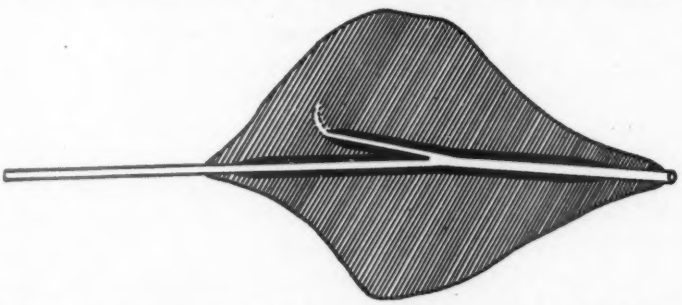
on examination with a very narrow diaphragm. When the elastic lamellae were destroyed, which was often the case, exact localization within the layers of the vessel wall was impossible (Figs. 6, 7, and 8). The homogeneous matter sometimes was encircled by the more or less intact elastic lamella (Fig. 5, early lesion?). Staining for metachromasia was not helpful in differentiation. The homogeneous matter surrounded the lumen entirely, or in part. It was symmetric, or much thicker on one side. Its outer contour was sometimes sharp, sometimes indistinct. The frequently found, scattered chromatin fragments indicated a fluid or semifluid state of the homogeneous areas. Occasionally, intact nuclei, resembling those of the surrounding inflammatory nodule, were present in the ring of necrosis. Since frozen sections were seldom available, not many sudan stains were made. They were negative, or weakly positive. The endothelium often was destroyed, but occasionally there was swelling with pyknotic or clear nuclei, and there was slight or marked overgrowth (Fig. 7). In one serially sectioned nodule, the endothelium showed little change throughout. The inflammatory nodule proper consisted of a loose or dense aggregation of cells with much variety in cell types (Fig. 6). Various mononuclear elements were found, some plasma-cell-like, some small and round, some spindle-shaped. Polymorphonuclear leukocytes and eosinophilic leukocytes occurred in varying numbers. In one focus, lobulated nuclei prevailed; another from the same paraffin block contained mostly cells with simple nuclei. The distribution of the cells, together with varying degrees of edema, made the lesion appear, in the stained slide, as a dark spot or as a clear one (Fig. 2), depending also on the character of the surrounding tissue. Most nodules were sharply outlined; others merged into adjoining structures. No giant cells were seen and no large cells with basophilic cytoplasm. No microorganisms were found.

The focal nature was easily recognized on longitudinal sections. There seldom was doubt whether a given lesion represented an arteritic focus. Adventitial edema around a small artery whose media was hyaline created a somewhat similar picture, but the similarity disappeared on closer inspection. Arteritis may be overlooked when the nodule is cut tangentially, not showing the center with the vessel wall. In no instance did the arteritis result in a morphologically discernible disturbance of tissue nutrition.

The largest foci (about 1 mm.) were visible to the naked eye in the stained slide; they were found in young and in older people (14 years, 19 years, 42 years). Coalescence of foci on tortuous vessels (Fig. 4) resulted in even larger areas. Many foci measured 0.25 to 0.3 mm. in

the axis of the vessel. None were exactly spherical. The thickness generally was one-half to two-thirds of the length, some being much thinner. One nodule was 470 μ long: the homogeneous change in the wall extended through 330 μ, the greatest thickness was 280 μ. Most foci measured less than 0.2 mm. Large and small foci, in general, occurred with equal frequency in the different layers of the appendix; the very small ones, perhaps, were found more often in the serosa or subserosa.

All possible variations in distribution were observed, but the lesions were more frequent in the tip than in the remainder of the appendix. No regularity existed except for this. Serosa, muscle coat, and submucosa might all be equally affected, or only one of them; there might be one nodule, or a few in the submucosa, while the serosa was full of them. Sometimes, serial sections of the block next to a positive slide failed to show lesions. They were evenly spaced in one portion, irregularly distributed in another. They were present in obliterated appendices and in patent ones; they sometimes were restricted to the patent segments, sometimes to the obliterated ones. The degree of involution of the appendix did not influence the findings significantly. Lesions which were obviously more recent were found in the patent segments of a partly obliterated appendix, but the opposite occurred as well. The direction of the arteries, whether radial, circular, or



Text-figure 1. Reconstruction by plotting 64 cross sections of an arteritic focus which occupied a fork. Length, 0.5 mm. Necrosis of the arterial wall is shown in solid black, inflammatory nodule is shown in oblique hatching. The necrosis extends much farther in one branch of the fork than in the other.

longitudinal, made no difference. Many lesions occupied a fork (Fig. 9 and Text-fig. 1). Three foci, belonging to the same vessel, were separated by stretches of normal artery only a fraction of a millimeter long. Small arteries of the mesoappendix occasionally were affected. Some foci were close to a carcinoid, even with carcinoid cells in the

arteritic nodule. (This is not astonishing since about every 40th appendix contained a carcinoid.) Relation to neuroma requires no discussion because at least one of 3 appendices showed a neuroma in the tip. The arteritic lesions, even when numerous, did not result in an inflammatory process of appreciable dimension. Scattered, non-characteristic, inflammatory areas in a few appendices were suggestive of hematogenous appendicitis; but no arteritis was found in these appendices by serial sectioning, and the hematogenous origin could not be proved.

Occurrence

Focal arteritis was found 88 times in 6,576 surgically removed appendices (1.34 per cent). Fifteen examples were found among the 1,930 specimens from male patients (0.78 per cent), 73 among the 4,646 appendices from females (1.57 per cent). A microscopic diagnosis of acute appendicitis was made in 2,282 cases; this group contained only 9 instances of focal arteritis. Four of these 9 occurred in 274 females of the second decade, 3 in the 269 males of the third decade, against only 2 for the 1,739 cases of acute appendicitis in all other age and sex groups.

Five hundred and fifty-six appendices had been removed in the course of operations on the biliary tract; 14 of these showed focal arteritis (2.5 per cent). For the 86 males in this group, the percentage was 3.49, for the 470 females it was 2.34. Ninety-six appendices came from pregnant women; 2 of them showed the lesion (2.1 per cent). Among 3,185 appendices removed during various gynecologic operations, 30 showed focal arteritis (0.94 per cent). The distribution among age groups (Table I) for males and females combined showed a low percentage for the fifth decade. In the third, fourth, and seventh decades there was no marked difference in the occurrence of focal arteritis between the sexes. In the second decade, however, there was only one instance of focal arteritis in 499 males (0.2 per cent) against 19 in 660 females (2.8 per cent); and in the sixth decade the corresponding percentages were 0.7 and 1.7.

One hundred and sixty-three appendices had been removed in the course of operations for hernia; 3 of the 4 cases of focal arteritis in this group were found in the 29 female patients with hernia, one each in the fourth, fifth, and sixth decades. But there was only one example of focal arteritis in the 134 appendices removed from males during operations for hernia. The average age for the cases of focal arteritis was 31 years, the average age for 1,500 appendectomy cases selected at random was 30½ years.

There was no seasonal variation and no relation to drugs.

TABLE I

Distribution by Age and Sex of Focal Arteritis of the Appendix

	0–9 yrs.		10–19 yrs.		20–29 yrs.		30–39 yrs.		40–49 yrs.		50–59 yrs.		60 yrs.		Total	Total	Total
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M and F
All appendices	235	136	499	666	390	1181	345	1303	255	998	132	288	74	74	1930	4646	6576
Focal arteritis	0	2	1	19	8	17	4	17	0	12	1	5	1	1	15	73	88
Focal arteritis (%)	0	1.5	0.2	2.8	2	1.5	1.2	1.3	0	1.2	0.7	1.7	1.4	1.4	0.78	1.57	1.34
Focal arteritis (%), both sexes	0.5		1.7		1.6		1.3		0.88		1.7		1.4				
Herniorrhaphies	2	0	16	4	22	4	20	5	36	8	27	6	11	2	134	29	163
Focal arteritis, herniorrhaphies	0	0	0	0	1	0	0	1	0	1	0	1	0	0	1	3	4
Operations on gallbladder	0	0	1	3	5	78	22	164	27	130	24	83	7	12	86	470	556
Focal arteritis, operations on gallbladder	0	0	0	0	1	2	1	3	0	3	1	2	1	1	4	11	15
Acute appendicitis	208	112	377	274	269	240	185	144	135	82	85	67	61	43	1320	962	2282
Focal arteritis in acute appendicitis	0	0	0	4	3	1	1	0	0	0	0	0	0	0	4	5	9

The question arose whether isolated arteritic foci might occur frequently in the appendix. Therefore, many cross sections and some longitudinal sections of 16 surgically removed appendices were studied; but even the search through hundreds of sections did not reveal arteritis. These specimens covered a wide range of ages and of pathologic conditions.

The appendices from 107 unselected autopsies were searched. In most cases, 20 to 70 sections were studied, 10 to 20 in others, occasionally 100 or more. Arteritis was found in 4 (female, 33 years, with hypertension, cerebral hemorrhage; female, 57 years, with rheumatic myocarditis; female, 43 years, with polycystic kidneys, pneumonia; male, 54 years, with chronic glomerulonephritis, coronary sclerosis). In 93 of these autopsies, the data for sex and age were available; 41 were between 30 and 60 years of age; 60 were males, 33 were females. Chronic cardiovascular diseases predominated in this material.

Focal arteritis was found only 6 times in organs other than the appendix. All 6 patients were females. An otherwise not remarkable tube and

ligament, removed during a Wertheim operation, contained many foci. (The uterus was not available.) Numerous foci were present in the completely occluded, partly ossified tubes of a 30-year-old obese woman. A single one stood out in the myometrium of the prolapsed uterus of a 48-year-old woman. One was found in a routine section of kraurosis vulvae, and serial sections showed a few more, all situated in uninflamed tissue. Arteritic foci were present in esophagus, liver, ovary, and in the fibrosed nephrectomy stump of a 63-year-old woman who had died of thrombocytopenic purpura of 4 months' duration. The hemorrhages were not related to the inflamed arteries. Once focal arteritis was found in the submucosa of the colon (female, 57 years, with old and recent rheumatic myocarditis, arteriolosclerosis of kidneys, ulcers of cecum). No arteritis of this type was found in the numerous single, multiple, or serial sections of other organs which were examined for other purposes during the years of this study. The predilection of focal arteritis for the appendix is beyond doubt. A special search was made, for obvious reasons, in several cases of severe rheumatic fever; no focal arteritis was found.

The absence of arteritis in the other organs could be proved in the autopsy material. The paucity of focal arteritis in other surgical material makes it highly probable that the patients who had focal arteritis in the appendix did not have it in other parts of the body. Organs that were removed during the same laparotomy were searched in a number of instances (5 uteri, 5 pairs of tubes, 14 ovaries, 11 gallbladders). No arteritis was found.

Comment

Either focal arteritis did not exist in previous decades, or it has escaped notice. The older literature about the vermiform appendix, including Aschoff's papers, does not mention arteritis as far as I know. Abrikossoff,[4] in 1935, wrote about hyperergic inflammation of blood vessels in appendicitis. His paper refers to a previous publication (in Russian). Small arteries showed fibrinoid swelling and necrosis of the wall, accompanied by karyorrhexis and perivascular infiltration. In another appendix, Abrikossoff found an artery with necrosis of the media and with perivascular histiocytic infiltration. His paper refers to a third observation made by Zlotnikoff. None of the three appendices showed active inflammation.

Scholl,[5] in 1936, examined nine pieces of the adherent, otherwise normal appearing appendix of a 20-year-old girl who had had appendicitic symptoms at times for 5 years. The mucosa was not inflamed.

Arteries in the submucosa, and one artery in the mesoappendix, showed arteritic foci with homogeneous media, swollen endothelium, and periarterial inflammation. Scholl concluded that this was either oligosymptomatic periarteritis nodosa, a residuum of appendicitis, or, finally, an arteritis sui generis. Neither Abrikossoff nor Scholl was familiar with previous American publications. Karsner's [6] monograph on arteritis contains a characteristic illustration; the appendix was only slightly inflamed, and there was no indication of arterial disease elsewhere in the body.

Evaluation of Figures

The greater frequency in females is significant at the 0.003 level. The fact that no male had focal arteritis in an organ other than the appendix, and that 6 females had such lesions is striking, but it cannot be evaluated statistically. For females of the second decade and males of the third decade, as compared with all other females or males, the levels of significance in the differences of frequency are 0.026 and 0.023. The predominance of females in the smaller group of appendices removed during operations for hernia is still suggestive at the 0.087 level. In the large group of acute appendicitis, with its low total incidence of focal arteritis, the females of the second decade and the males of the third decade predominate significantly (0.01 level).

In spite of the large number of appendices studied, the figures for some specific groups are too small for accurate statistics. Some, however, are impressive. When, for instance, only 2 cases of focal arteritis were found among 218 appendices from girls between the ages of 10 and 14, while 448 appendices from girls between 15 and 19 yielded 17 cases, it is difficult not to believe that there is a biologic basis for this difference which is significant at the 0.01 level (Table II). Corresponding subdivision of other decades showed no marked differences.

Table II

Difference in Frequency of Focal Arteritis in First and Second Half of Second Decade

	10–14 yrs.		15–19 yrs.	
	M	F	M	F
Appendices	242	218	257	448
Focal arteritis	0	2	1	17
Focal arteritis (%)	0	0.92	0.39	3.8

A few more figures might be mentioned without attempts at interpretation. A predominance of females with appendiceal arteritis was not evident in the patients who had had gallbladder operations. No focal arteritis was found among 117 specimens removed for acute

appendicitis in people over 40 years of age, but there were 8 examples among 1,160 young patients (from 10 to 29 years) with appendicitis. The distribution among age groups was much more even in gynecologic patients. The relative rarity of focal arteritis in patients with acute appendicitis is in contrast with its frequency in those having operations upon the biliary tract. One must consider, however, that focal arteritis can be overlooked more easily in an inflamed appendix.

Focal arteritis occurs at all ages; it is much more frequent in the female than in the male. It is not significantly connected with other diseases, so far as the material available allows us to judge. The distribution as to age and sex sets focal arteritis apart from periarteritis nodosa. In the latter, males predominate (3:1), and 50 per cent of the patients are in the fourth and fifth decades.[7] The average age, correspondingly, is higher than in focal arteritis (37 years).[8]

If focal arteritis were closely related to periarteritis nodosa, we should expect to see it frequently in the appendices of people who have died of periarteritis nodosa. Most reports on that disease do not mention the appendix. In 30 necropsies at the Mayo Clinic,[9] the appendix was found to be involved in 2. In the same series, arteritic lesions were found 3 times in the rectosigmoid, 6 times in the remainder of the colon, 7 times in the ileum, 4 times in the duodenum, and 6 times in the stomach. This shows that the arteries of the appendix are not more frequently affected by periarteritis nodosa than arteries in other parts of the gastro-intestinal tract. In one series of 7 cases,[10] one showed arteritis in the appendix. Of these 7 cases, one showed arteritis in the colon, 2 in the ileum. There are additional case reports of periarteritis nodosa with lesions in the appendix.[11-14] No exhaustive search of the literature has been made concerning this point, but the figures mentioned appear sufficient to show the difference in this respect between focal arteritis and periarteritis nodosa. Focal arteritis, in my opinion, cannot be considered a mild form of periarteritis nodosa.

It has been assumed that the different forms of arteritis are related to each other.[15,16] Jäger [15] described periarteritis-nodosa-like lesions in thromboangiitis. Two of his descriptions might refer to focal arteritis (the appendix is not mentioned). Periarteritis nodosa developed in the arm of a 20-year-old soldier who suffered from chronic suppuration after being shot in the hand. The main lesion in his brachial arteries, however, was an obliterating endarteritis.[17] Rossier [16] mentioned that many diagnoses of periarteritis nodosa have been made during microscopic examination of the appendix, removed incidentally in the course of an operation. It appears hazardous to diagnose this disease from

the appendix, since the single lesions of periarteritis nodosa and of focal arteritis may be indistinguishable from each other. It has come to my knowledge repeatedly that the finding of arteritis in a surgically removed appendix has aroused the fear of a fatal disease like periarteritis nodosa. Cases of focal arteritis have been reported as periarteritis nodosa (*e.g.*, cases 2 and 3 of Spiegel).[18] One of my old observations has been mistakenly included, by others, in a discussion on periarteritis nodosa.[19]

The morphologic pattern of arteritis is fairly constant. Therefore, it is understandable that some of the necrotizing arterial foci that occur in laboratory animals resemble focal arteritis. I do not recall that any of these lesions, in treated or in untreated animals, were restricted to a single small organ as is focal arteritis.

An attempt at explaining focal arteritis, especially its restriction to one small part of the intestine, appears hopeless in the present state of our knowledge. The difficulties, however, are not much more formidable than for other forms of arteritis, since many are more or less focal. The postulated local factor, that leads to circumscribed fibrinoid necrosis of the arterial wall, can be active in an arterial system that appears normal; this is the case in focal arteritis. But, on the other hand, I have seen a single focus of necrotizing arteritis of a large suprarenal branch, while the arteries in many other organs showed multiple thrombi without inflammation. The non-specific necrotizing arteritis that is found occasionally in various kinds of generalized infection also has its unexplained preferences, namely, the testicle, the fat tissue near the kidney pelvis, and around the suprarenal gland. Why certain reactions between the vessel wall and the fluid that is pressed into it take place at one point and not at another, is a general problem.

It would be out of keeping with modern biologic thinking to look for "the cause" of focal arteritis; we must search for the conditions that bring it on. Since it occurs in otherwise healthy people and in association with so many different diseases, one is led to think of some general principle like stress. Arteritic lesions provoked experimentally by one adrenocortical hormone can be made to heal by another.[20] This may help us in understanding the benign character of focal arteritis. Another general principle comes to mind. Non-antigenic substances, as Landsteiner and van der Scheer have shown,[21] can become antigenic by combining with protein. The finding of focal arteritis in the mesentery of the appendix shows that the physical conditions prevailing in this structure are not a decisive factor in the genesis of focal arteritis. The mysterious process of involution and obliteration of

the appendix is not definitely connected with focal arteritis, although the lesion is more frequent in the obliterated tip. The assumption of "chronic appendicitis" (which I believe to be a rare disease) as an etiologic factor would not be helpful. Abdominal organs, like the gallbladder or the fallopian tube, which often are the seat of undisputable long-standing or recurring inflammation, do not show arteritis. One cannot say that the arteries of the appendix are, in general, more susceptible than other vessels. In a case of rapidly progressing malignant sclerosis, for instance, the arteries in different portions of the intestine were severely diseased, but those in the appendix were spared. Thus, the idea of the appendix, as a rudimentary organ, representing a point of lower resistance is not helpful in explaining focal arteritis. The coexistence, even in the same section, of recent and of older lesions indicates that some causative mechanisms are active for a certain time, or at intervals. The duration of the disease is unknown. One might estimate the age of the lesion of focal arteritis in its full development at 3 to 6 weeks by comparing with experimental lesions of known age [22]; the reliability of such a comparison is doubtful.

No characteristic scar has been recognized. Small arteries in the appendix become hyaline so frequently that the scar of an arteritic process cannot be expected to be distinguishable.

If we knew something about the geographic distribution of focal arteritis, a clue to its causation might be provided; perhaps some substance in the modern food industry is responsible. That would explain the lack of older observations. A dietary factor seemed to be active in the arteritis observed in rats by Wilens and Sproul.[23] Prevention of arteritis by vitamin E is an even stronger hint in this direction.[24] There was, as mentioned, no connection in my series between focal arteritis and medication.

Sometimes severe focal arteritis was the only lesion in an appendix when the clinical picture had been that of acute abdominal distress. These patients were free of abdominal symptoms after appendectomy. Nevertheless, it is not admissible to correlate the abdominal symptoms and the appendiceal arteritis. Even the severe, widespread inflammation of abdominal arteries, as it occurs in periarteritis nodosa, could not be correlated with abdominal symptoms in 30 autopsied cases.[9] The inextricable psychosomatic complex of abdominal pain, especially in young females, can often be cured by laparotomy. In the first years of this study, the patients were followed. When it was recognized that no clinical correlation with focal arteritis existed, the follow-up study was discontinued. Moschcowitz [25] stated that "time alone will decide"

whether these patients who have appendiceal arteritis will remain well. The past 22 years have made this decision, in my opinion.

As long as its genesis is unknown, focal arteritis might be included in Karsner's group of primary arteritis.[6]

Summary

Foci of necrotizing arteritis with surrounding inflammation were found in 88 of 6,576 appendices (1.34 per cent). This focal arteritis occurred more frequently in females than in males. The peak of frequency for females was between the 15th and 20th year; that for males between the 20th and 29th. The lowest percentage for adults of both sexes was found in the 5th decade. Focal arteritis is practically restricted to the vermiform appendix, being found very seldom in other organs. Focal arteritis is different from periarteritis nodosa in every respect except the morphologic picture. Its genesis is unknown. No clinical correlation could be established.

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DESCRIPTION OF PLATES

The patients from whose appendices the photomicrographs were made had no symptoms of vascular disease or of generalized infection.

All photomicrographs are from sections stained with hematoxylin and eosin, unless otherwise stated.

PLATE 43

FIG. 1. Typical arteritic focus in the submucosa of the appendix. The submucosa is fibrosed and, in places, supplanted by fat tissue. There is no other inflammation.

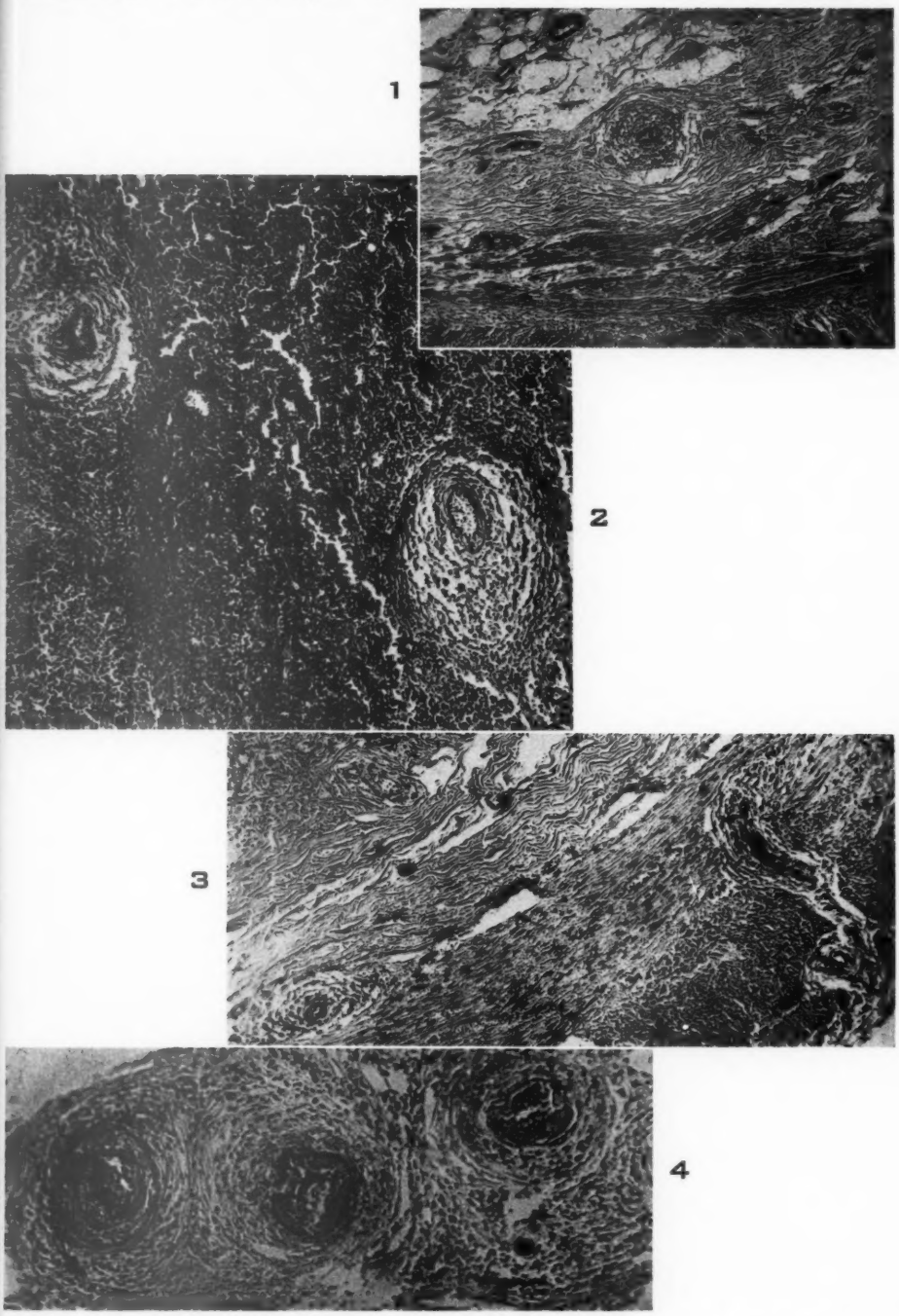
FIG. 2. Two edematous, arteritic foci in lymphoid tissue. The focus in the left upper corner has been cut through the area of necrosis.

FIG. 3. One focus, belonging to a perforating artery, is cut lengthwise; another, between the inner muscle coat and the submucosa, is seen in cross section.

FIG. 4. Tortuous artery with three cross sections showing arteritis. The internal elastic lamella is more or less preserved. Lithium-carmine and Weigert's stain for elastic tissue.

A

F



Plaut Focal Arteritis of the Appendix

PLATE 44

FIG. 5. Typical cross section of focal arteritis. The lumen is preserved; it is encroached upon by large nuclei, probably belonging to the endothelium. A thick, homogeneous layer separates the endothelium from the internal elastic lamella. The inflammatory nodule repeats the outline of the vessel proper. The media is hardly recognizable. The nodule presses against the inner muscle coat of the appendix. Lithium-carmine, Weigert's stain for elastic tissue, picric acid.

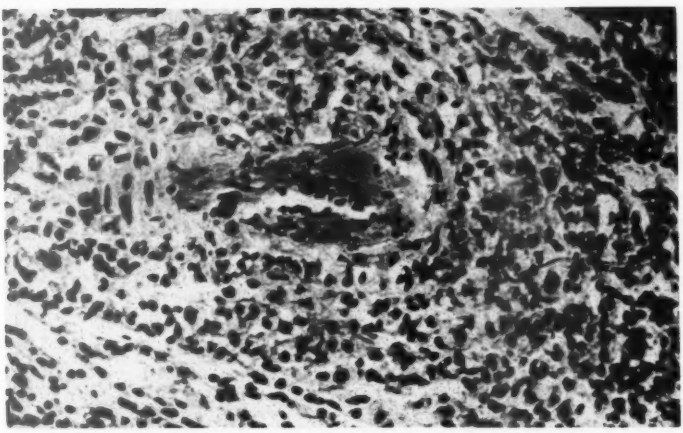
FIG. 6. Arteritic focus, probably an early phase. The nodule is loose; there is much variety in the shape of the nuclei. The area of necrosis is asymmetric.

FIG. 7. Central portion of Figure 6 at a higher magnification. Of note are the almost complete destruction of the media and the breaking up of nuclei.

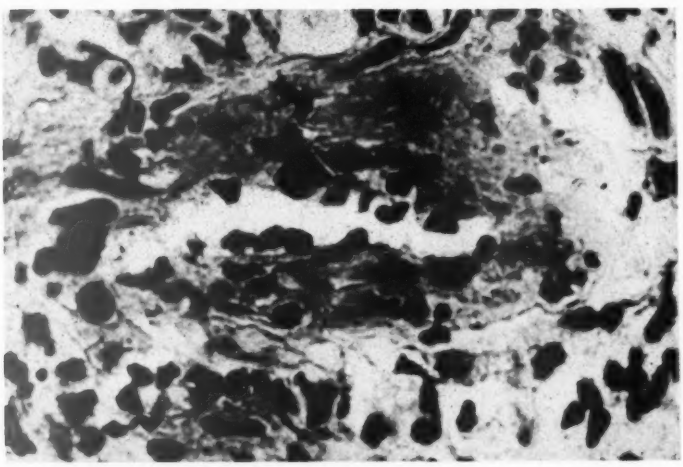
A

5

6



7



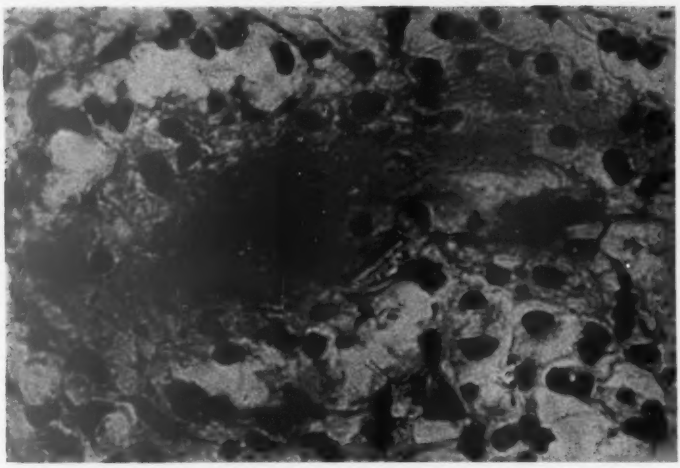
Plaut Focal Arteritis of the Appendix

PLATE 45

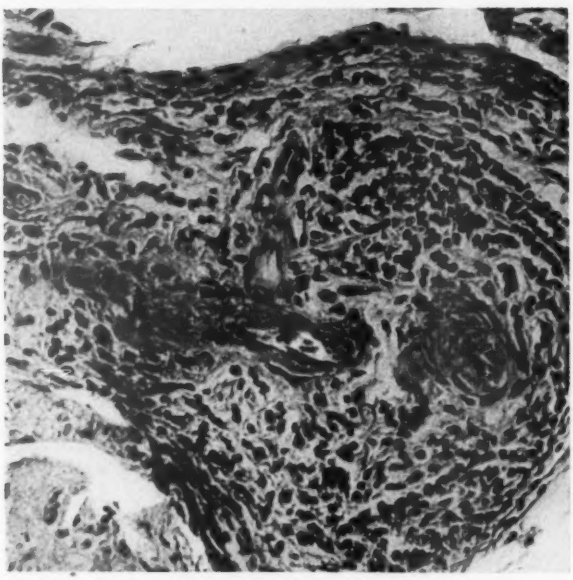
FIG. 8. Complete necrosis of the vessel wall.

FIG. 9. The artery has been cut beyond a fork with one branch in cross section, the other in oblique section. Arteritic foci are often located at forks.

8



9



Plaut Focal Arteritis of the Appendix

THE CELL TYPE OF SECONDARY PARATHYROID HYPERPLASIA *

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Secondary parathyroid hyperplasia was first described in 1914 by Marine [1]; the hyperplasia was produced in fowls by placing them on a low calcium diet. Hyperplasia following low dietary calcium also was reported by Luce [2] in 1923 and Stoerk and Carnes [3] in 1945. Secondary enlargement of the parathyroid glands was found to occur in rickets by Pappenheimer and Minor [4] (1921) and in pregnancy by Bodansky [5] (1939). In 1934 Albright, Baird, Cope, and Bloomberg [6] suggested that hypertrophy of the parathyroid gland may result from renal disease. In 1935 Pappenheimer and Wilens [7] supported this hypothesis by comparing the weights of the parathyroid glands from nephritic and non-nephritic patients. They found a definite increase in the weights of all four parathyroid glands in patients with nephritis, the amount of enlargement depending upon the severity of the renal insufficiency. Parathyroid hyperplasia secondary to renal damage was finally established as an entity in 1937 by Albright, Drake, and Sulkowitch [8] who described the salient feature of "renal osteitis fibrosa cystica." The resultant pathologic changes occurring in the parathyroid glands were described by Castleman and Mallory [9] who studied the parathyroid glands from 29 patients with chronic renal insufficiency. Because the predominant cell in the majority of their cases was the normal-sized chief cell, the parathyroid enlargement was termed chief cell hyperplasia. This they contrasted with *wasserhelle* cell or primary hyperplasia, a condition in which all four parathyroid glands are enlarged, apparently in the absence of renal or other diseases known to cause secondary parathyroid hyperplasia.

Since 1937, the cell type has been used as a criterion for distinguishing between primary and secondary parathyroid hyperplasia when all four glands were involved. Albright, Sulkowitch, and Bloomberg [10] reported 6 cases of "hyperparathyroidism due to idiopathic hypertrophy of parathyroid tissue." The histologic picture of *wasserhelle* cell hyperplasia in these cases was an important factor in arriving at a diagnosis of primary, instead of secondary, hyperplasia. The same interpretation was made by Rogers, Keating, Morlock, and Barker,[11] who reported 3 additional cases of "primary" hyperplasia and stated: "So far as is

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known, hyperplasia of the parathyroids involving *wasserhelle* cells is always associated with primary hyperparathyroidism."

Ginzler and Jaffe [12] found chief cell hyperplasia of the parathyroid glands in 12 cases of renal insufficiency. Nelson,[13] however, stated that secondary parathyroid hyperplasia may be of any cellular type. Bell [14] also believed that the cell type is a variable histologic feature in secondary hyperplasia, consisting of vesicular and water-clear cells in addition to chief cells. Norris [15] stated that this type of hyperplasia is characterized by the presence of increased numbers of vesicular cells, and Pappenheimer and Wilens,[7] in their study of secondary hyperplasia, found that the dominant cell was the water-clear cell. Castleman and Mallory [9] determined the chief cell to be predominant in severe cases of renal disease, but found in milder cases that the parathyroid glands consisted of "chief cells of the transitional *wasserhelle* type." It is apparent that the cell type of secondary parathyroid hyperplasia has not been clearly established.

The purpose of this paper is to report the results of a study of the parathyroid glands in 61 cases of bilateral renal disease, with particular emphasis on the predominant cell type.

Method

The material for this study was obtained from a series of 137 autopsies. The skin of the neck was reflected to the cricoid cartilage and the ribbon muscles separated, exposing the deeper structures of the neck. By sharp block dissection the thyroid gland, trachea, esophagus, and immediately surrounding tissue were removed, leaving the carotid arteries intact. The parathyroid glands were then dissected from the posterior fascia of the thyroid gland and placed in a 10 per cent formalin solution. After several days of fixation all surrounding adipose and connective tissue was removed and each gland weighed. The glands were dehydrated, imbedded in paraffin, sectioned longitudinally at a thickness of 6 μ, and stained with hematoxylin and eosin.

The 137 cases were divided into two groups. In group I were placed all cases in which there was definite anatomical evidence of bilateral renal disease. All others were placed in group II.

Results

Weight

The mean weight per parathyroid gland was 75.2 mg. in the 61 cases of group I and 42.6 mg. in the 76 cases of group II. In 43 cases (70 per cent) of group I, the mean weight per gland was greater than 42.6 mg.

The remaining 18 cases (30 per cent) included the following types of renal disease:

Mild arteriolosclerosis	8 cases
Acute pyelonephritis	5 cases
Severe arteriolosclerosis	2 cases
Subacute glomerulonephritis	1 case
Intercapillary glomerulosclerosis	1 case
Congenital polycystic kidneys	1 case

In the 43 cases of group I in which there was hyperplasia of the parathyroid glands, each of the four glands in each case weighed approximately the same, the weight being proportionate to the severity and duration of the renal disease. The greatest increase in weight was found in a case of long-standing severe renal arteriolosclerosis, the mean weight per gland being 213 mg.

In group II the parathyroid glands were soft and dark yellowish brown. In group I the parathyroid glands were usually light creamy gray, firm, and slightly lobulated, this appearance becoming more marked as the weight increased.

Histologic Appearance

For each case the histologic appearance of all four glands was practically identical. There was less adipose tissue in the parathyroid glands of group I, the decrease appearing to be actual as well as relative, varying directly with the severity and duration of the renal disease and with the increase in weight of the glands.

The predominant cell in 32 cases of group I was the clear cell, a large, polyhedral or round cell, measuring between 15 and 20 μ in diameter. The nucleus was small, dark, and eccentrically located, and the cell membrane was distinct. Usually the cytoplasm was absolutely clear, but in a few cells it contained faint, granular, eosinophilic material. The size was not constant; in the more advanced cases the cells were of the large, typical *wasserhelle* type. In the more hyperplastic glands there was a tendency toward an alveolar arrangement, with the nuclei situated in the basilar portions of the cells. This, however, was not an outstanding feature. In none of these cases was there an increase in oxyphilic cells and, in many, oxyphilic cells were absent. The 32 cases in which this cell type was found included the following renal diseases:

Severe renal arteriolosclerosis	10 cases
Chronic pyelonephritis	9 cases
Chronic glomerulonephritis	6 cases

Mild renal arteriolosclerosis	3 cases
Congenital polycystic kidneys	2 cases
Subacute glomerulonephritis	1 case
Sulfonamide nephropathy	1 case

In 23 cases of group I, the parathyroid glands contained chief cells of normal size, transitional cells, and clear cells. Transition from normal-sized chief cells to vesicular cells and then to clear cells could be demonstrated. These 23 cases included the following types of renal disease:

Mild renal arteriolosclerosis	8 cases
Acute pyelonephritis	7 cases
Severe renal arteriolosclerosis	5 cases
Intercapillary glomerulosclerosis	1 case
Chronic pyelonephritis	1 case
Chronic glomerulonephritis	1 case

In the remaining 6 cases of group I, the normal-sized chief cell predominated. Renal disease of the following types was encountered in these cases:

Mild renal arteriolosclerosis	3 cases
Acute pyelonephritis	2 cases
Severe renal arteriolosclerosis	1 case

In only 6 cases of group II was the vesicular or clear cell predominant. In none of these cases was there an increase in weight of the parathyroid glands. In 22 cases of group II, normal-sized chief cells and clear cells were present. Normal-sized chief cells were predominant in the remaining 48 cases of group II.

These findings are summarized in Table I.

Discussion

Three cell types are recognized in this discussion, *viz.*, chief cells, vesicular cells, and clear cells. The vesicular cell is an intermediate type, representing partial clearing. The degree of vesiculation is proportionate apparently to the severity of hyperparathyroidism. In this paper the term clear cells frequently includes vesicular cells for brevity inasmuch as the latter represent partial or incomplete clearing and therefore constitute a change of the same character.

Pappenheimer and Wilens [7] found that the degree of secondary parathyroid enlargement is correlated with the severity of the renal disease. This correlation was substantiated by the present study. In those cases in which the renal disease was severe and of long duration (severe renal

arteriolosclerosis, chronic pyelonephritis, and chronic glomerulonephritis), there was an increase in the mean weights of the parathyroid glands. If the renal disease was mild or of short duration (mild renal arteriolosclerosis, acute pyelonephritis, subacute glomerulonephritis), there was no increase in the mean weight of the parathyroid glands (Table I). There usually is hypertrophy of the parathyroid gland in

TABLE I

Summary of Findings in the Parathyroid Glands of 61 Patients With, and 76 Without, Renal Disease

Renal disease	No.	Mean weight	Chief cell predominant	Chief and clear cells	Clear cell predominant
		mg.			
None (group 2)	76	42.6	48 (63%)	22 (29%)	6 (8%)
All types (group 1)	61	75.2	6 (9%)	23 (38%)	32 (53%)
Severe renal arteriolosclerosis	17	97.8	1 (6%)	5 (29%)	11 (65%)
Mild renal arteriolosclerosis	13	40.0	3 (23%)	8 (61%)	2 (16%)
Chronic pyelonephritis	10	86.7	0	1 (10%)	9 (90%)
Acute pyelonephritis	9	43.6	2 (22%)	7 (78%)	0
Chronic glomerulonephritis	7	121.8	0	1 (14%)	6 (86%)
Congenital polycystic kidneys	2	61.0	0	0	2 (100%)
Subacute glomerulonephritis	1	35.0	0	0	1 (100%)
Intercapillary glomerulosclerosis	1	38.0	0	1 (100%)	0
Sulfonamide nephropathy	1	83.0	0	0	1 (100%)

severe, long-standing renal disease. Castleman and Mallory [9] observed that in secondary hyperplasia the parathyroid glands tend to become creamy gray, firm, and lobulated. These changes were found in all cases of renal disease in the present series in which there was definite parathyroid enlargement. The number of fat cells in the parathyroid glands steadily decreases with the degree of hyperplasia, as was demonstrated in the present study by comparing low-power camera lucida tracings of hyperplastic glands with similar tracings of normal glands.

From the figures in Table I it is evident that the predominant cell in the majority of cases without renal disease was the normal-sized chief cell (63 per cent), whereas in the majority of cases with renal disease it was the vesicular or clear cell (53 per cent). The preponderance of clear cells is more striking if only those cases are included in which the renal disease was chronic and severe (severe renal arteriolosclerosis, 65 per cent; chronic pyelonephritis, 90 per cent; chronic glomerulonephritis, 84 per cent). In addition, the clear cell was the predominant type in both cases of congenital polycystic kidneys and in the cases of subacute glomerulonephritis and sulfonamide nephropathy. In no form of renal disease was the normal-sized chief cell predominant in a majority of cases. That the usual predominant cell of secondary parathy-

roid hyperplasia is the clear cell supports the opinion of Norris [16] that the clear cells are the most active physiologically.

In Table I it is shown that in those cases of renal disease in which the clear cell was not predominant, the parathyroid cells usually were divided between chief cells and clear cells (mild renal arteriolosclerosis, 61 per cent; acute pyelonephritis, 77 per cent; intercapillary glomerulosclerosis, 100 per cent). This seems to indicate that with mild renal disease, or renal disease of short duration, there is a transition from normal-sized chief cells to the more physiologically active clear cells.

Pappenheimer and Wilens,[7] in studying the parathyroid glands in cases of renal disease, found that oxyphilic cells were not increased in number. In no case of renal disease in the present study was there an increase in oxyphilic cells.

The clear cells vary in size and in some instances may become large *wasserhelle* cells, considered by some to be diagnostic of primary parathyroid hyperplasia. However, the presence of these cells cannot be used as a criterion for establishing the diagnosis of primary parathyroid hyperplasia. In making a diagnosis of primary hyperplasia, renal insufficiency resulting from nephrolithiasis due to parathyroid adenoma must be ruled out. Examples of combined hyperparathyroidism and secondary parathyroid hyperplasia have appeared in the literature. Lober, Hertzog, and Rice [17] reported a typical example of generalized osteitis fibrosa cystica in which a parathyroid adenoma was removed at operation, the patient expiring in uremia 3 years later. At necropsy, calcium deposits were found throughout the parenchyma of both kidneys and the remaining parathyroid glands were composed mainly of *wasserhelle* cells. Johnson [18] reported an almost identical case and stressed the progressive clearing of the cytoplasm of the chief cells of the secondarily hyperplastic parathyroid glands. Similar cases have been reported by Downs and Scott [19] and by Soffer and Cohn.[20]

The present study included 6 cases with no anatomical evidence of renal disease, in which the majority of parathyroid cells were clear cells. In 2 of these cases the patients had advanced portal cirrhosis. Perhaps this evidence of increased parathyroid activity can be explained on the basis of low dietary calcium. Marine [1] demonstrated parathyroid hypertrophy in fowls on a low calcium diet and Stoerk and Carnes [3] found the same to be true in rats placed on a low calcium diet. Another patient in the group of 6 had a chronic gastric ulcer. Possibly the hyperplasia in this case may be explained on the basis of long-standing alkaline therapy with resultant lack of calcium absorption. In the remaining 3

cases no explanation can be suggested for the predominance of clear cells.

Summary

The parathyroid glands were studied in 61 cases of bilateral renal disease. Seventy-six cases without renal disease provided control material. A definite increase in weight of the parathyroid glands was found in the case of renal insufficiency. This was proportionate to the severity and duration of the renal damage. A decrease in fat content of the hyperplastic parathyroid glands also was found. The predominant parathyroid cell in cases of severe, long-standing renal disease was the clear cell. In the majority of cases of mild renal disease or renal disease of short duration, the parathyroid glands contained chief cells and clear cells. Transitions between the two types could be seen. Oxyphilic cells were found not to be increased. The parathyroid clear cell is usually predominant in secondary parathyroid hyperplasia.

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DESCRIPTION OF PLATES

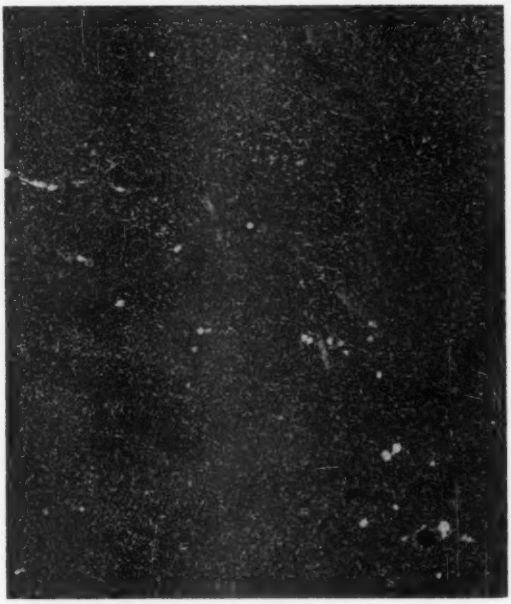
PLATE 46

FIG. 1. Lateral view of structures of the neck, showing secondary hyperplasia of the parathyroid glands from a case of severe nephrosclerosis. The glands are pale and lobulation is present. The mean weight was 213 mg.

FIG. 2. Parathyroid gland from a case of chronic pyelonephritis. The cells are compactly arranged and increased in number. The adipose tissue is diminished. × 30.

FIG. 3. Normal parathyroid gland. The predominant cell is the chief cell with a round, central nucleus and indistinct cell membrane. An area of adipose tissue is present. × 465.

1



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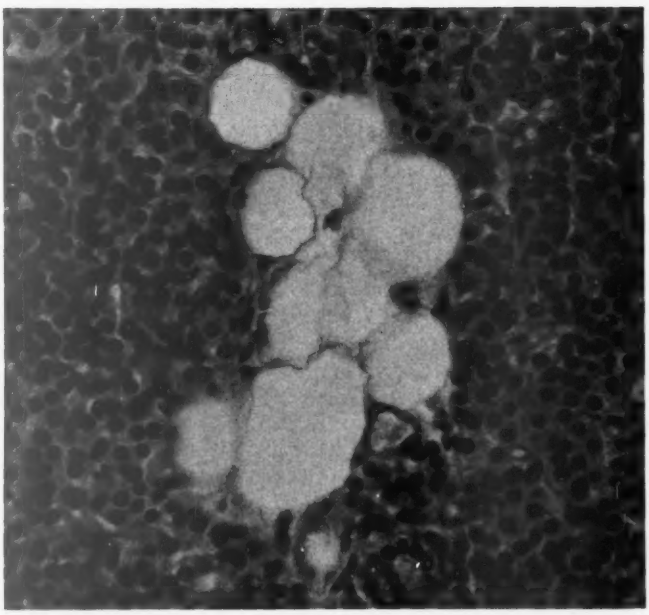


3

Fritz and Brines Secondary Parathyroid Hyperplasia

PLATE 47

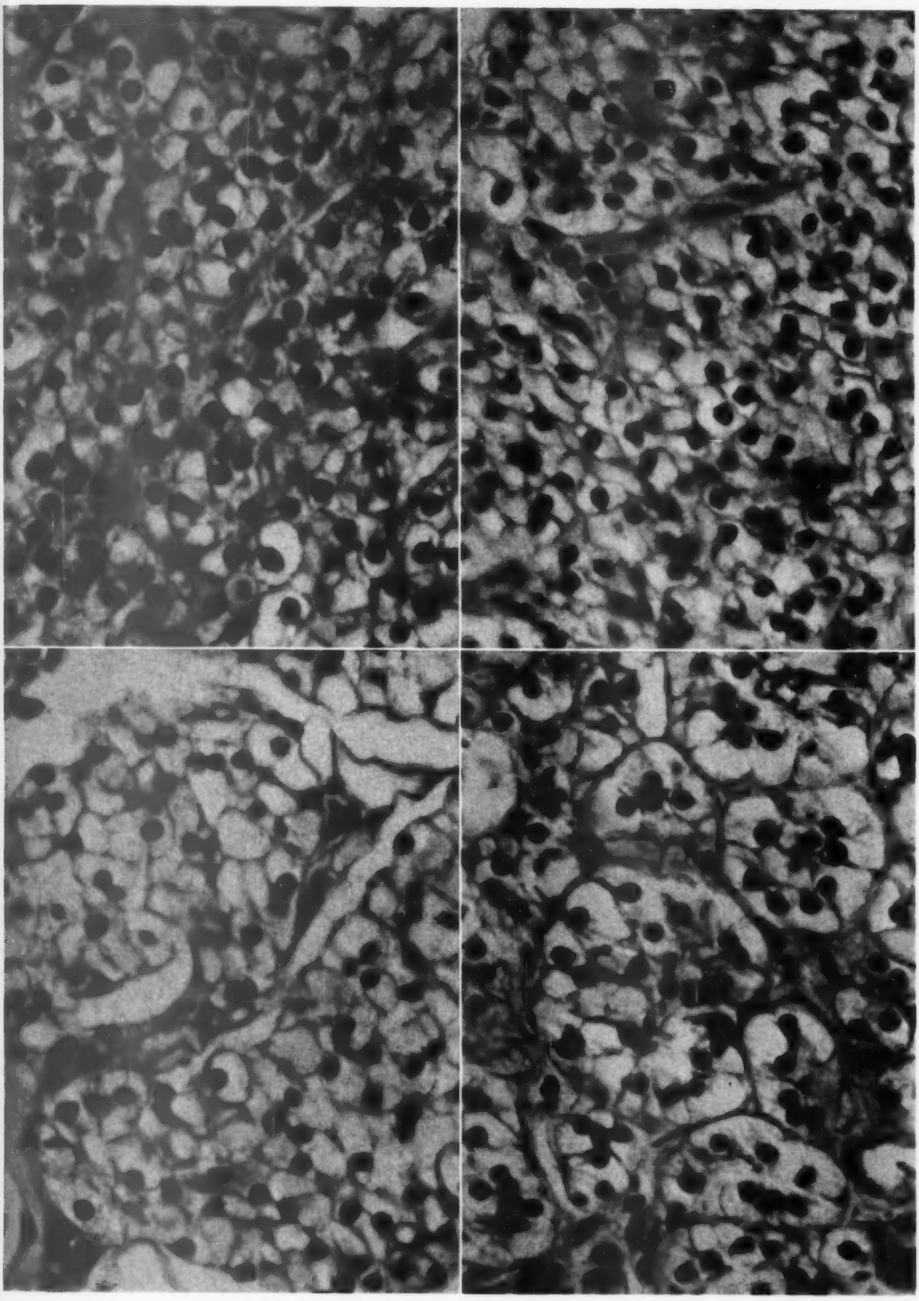
FIG. 4. Parathyroid gland from a case of chronic glomerulonephritis. Clear cells with eccentric nuclei, clear cytoplasm, and distinct cell membranes are predominant. A few chief cells are present also. There is a transition from chief to clear cells shown by the presence of vesicular cells with vacuolated cytoplasm. × 465.

FIG. 5. Parathyroid gland from a case of chronic pyelonephritis. Clear cells are predominant but vesicular cells are present also. × 465.

FIG. 6. Parathyroid gland from a case of nephrosclerosis. Clear cells are predominant. × 465.

FIG. 7. Parathyroid gland from a case of nephrosclerosis. Clear cells are predominant and there is a tendency toward an alveolar arrangement. × 465.

4 6



5 7

Fritz and Brines Secondary Parathyroid Hyperplasia

ALLERGIC GRANULOMATOSIS, ALLERGIC ANGIITIS, AND PERIARTERITIS NODOSA *

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During the past 25 years a relation between allergic states and vascular lesions of the type seen in periarteritis nodosa has been firmly established by study of human disease and by experimental evidence. Gruber [1] suggested that periarteritis nodosa is a hyperergic vascular response to infection. Cohen, Kline, and Young [2] postulated a causal relationship between allergy, as exemplified by severe asthma, and periarteritis nodosa developing in its course. Wilson and Alexander,[3] on reviewing the literature, found association of these two diseases in 18 per cent of 300 cases of periarteritis nodosa. Cases of this type have been studied also by Rackemann and Greene,[4] who described the clinical syndrome in some detail, and by Harkavy,[5] who interpreted this syndrome as an expression of vascular allergy. Rich [6,7] and other authors [8] found vascular lesions in patients who developed reactions to foreign serum or drugs (sulfonamides, iodine, dilantin), which Rich believed to be identical with those of periarteritis nodosa. Rich and Gregory [9] were able to reproduce these lesions experimentally in animals.

While most of the authors concerned themselves with pathologic changes in the vascular system, there are a number of reports in the literature indicating that, in addition, widely dispersed extravascular lesions may occur in connective tissue (Rössle,[10] Bergstrand,[11] Rich,[12] Smith [13]). Such lesions are characterized by inflammatory exudate rich in eosinophilic leukocytes, by necrosis of the exudate, and by severe alteration of collagen with granulomatous (epithelioid and giant cell) reaction.

The present report deals with cases of severe asthma which presented the clinical syndrome described by Rackemann and Greene,[4] and by Harkavy,[5] and which, on pathologic examination, exhibited the granulomatous extravascular lesions noted above, as well as necrotizing, inflammatory, and granulomatous vascular changes.

Most of our material is derived from cases autopsied in the Department of Pathology of the Mount Sinai Hospital, New York City. Of 23 patients in whom death was accompanied, or preceded, by severe asthma (status asthmaticus), 9 showed a strikingly uniform clinical

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picture. In addition to asthma, there were fever and hypereosinophilia, and symptoms of cardiac failure, renal damage, and peripheral neuropathy resulting from vascular embarrassment in various systems of organs. A patient with a similar syndrome was autopsied at the Long Island College Hospital * and another at the Barnert Memorial Hospital.* In addition, 2 patients with this syndrome are still alive and are being followed at the Mount Sinai Hospital. One patient, who later died at home, was treated at the Mount Sinai Hospital in 1941; at that time a specimen of a skin nodule was taken. No autopsy was obtained on this patient. Of the 14 cases exhibiting the clinical syndrome, specific anatomical lesions were found in 13. These 13 cases form the basis of the present report.

Clinical Data

Review of the data of our 13 cases (9 female, 4 male) yields a rather uniform clinical picture.

The age at the onset of asthma varied from 7 to 58 years. This preceded the terminal illness by an average of 3 years, with variation from a few months to 10 years. The course of the asthma was that of rapidly increasing severity until fever and hypereosinophilia appeared. Although one patient actually died in status asthmaticus, in the majority symptoms of asthma abated and sometimes completely disappeared during the course of the terminal illness.

Several patients exhibited sensitivity to various allergens and, sometimes, to bacteria cultured from the paranasal sinuses. Only a few reported a family history of allergy. Infection of the paranasal sinuses was present in the majority of cases; *Staphylococcus aureus, Staphylococcus albus,* and Pneumococcus were isolated from sinus washings in some of these. In one instance (M. M.) a pneumococcus was found in the sputum as well, during attacks of migratory pneumonia. In another instance (R. E.) blood cultures yielded a gram-negative rod during the initial stages of fever, identified as *Haemophilus para-influenzae.*

The duration of the terminal illness in the fatal cases varied from 3 months to 5 years. One patient is still alive 5½ years, and another, 1½ years after the onset of fever and eosinophilia.

Fever was present in all cases. It was very irregular; in some patients the temperature reached 40° C., but in the majority did not exceed 39°, or even 38° C. Usually the fever abated some time before death. There was marked leukocytosis, occasionally as high as 60,000 per cmm., and intense eosinophilia reaching 84 per cent of the total white count. Table I gives the absolute eosinophil count rather than percentage fig-

* We wish to thank Dr. Jean Oliver, and Dr. L. G. Shapiro and members of his staff for permission to publish these 2 cases.

Clinical Data in 13 Cases of Allergic Granulomatosis

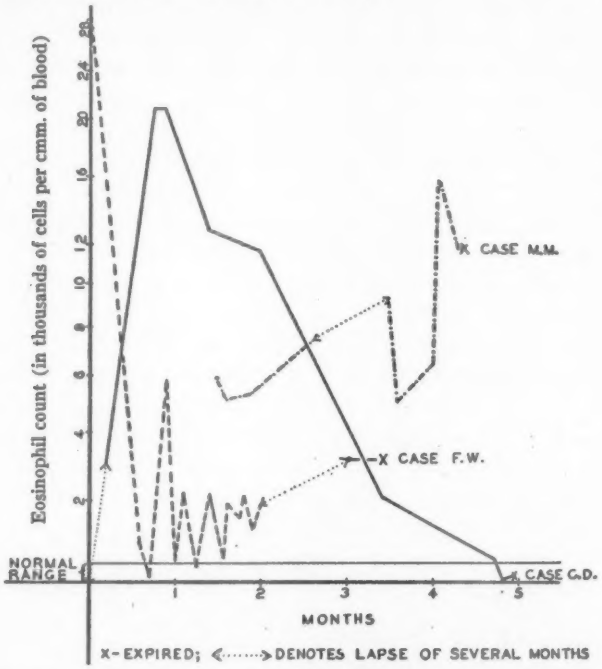
Case	Sex	Age at the time of death	Age at the onset of asthma	Evidence of sensitization	Family history of allergy	Sinusitis and other infections	Duration of terminal illness	Range of eosinophilia	Highest blood pressure	Organ manifestations									Cause of death	Remarks
										Lungs	Heart	Gastro-intestinal tract	Kidneys	Skin, purpura	Skin, nodules	Lymph-adenopathy	Peripheral neuropathy	Central nervous system	Joints	
		yrs.	*yrs.*				*mos.*	*cells/cmm.*	*mm. Hg*											
S.W.	M	38	35	+	o	+	3	90–8,100	120/90	+P	+Fl	+	o	+	o	o	+	o	o	Perforated peptic ulcer	
C.D.	F	45	44	o	o	o	6	60–21,500	150/110	+P	+Fl	+	±	o	+	+	+	+	o	Cardiac failure	BUN, 75
S.S.	F	9	7	+	+	+	16	1,200–19,600	168/120	+P	+Fl	+	+	+	+	+	o	+	o	Cardiac failure	
B.Z.	F	28	18	+	o	+	8	200–6,500	150/11	+PL	+	+	+	o	+	o	+	+	o	Uremia	BUN, 106
F.H.	F	24	18	o	o	+	60	0–29,000	136/90	+PL	+Fl	+	±	+	+	+	o	o	+	Cachexia	
H.C.	F	55	47	o	o	+	10	0–5,000	150/90	+PL	o	+	+	o	o	o	o	+	o	Status asthmaticus	
M.M.	F	23	18	+	+	+	24	5,000–15,700	182/128	+PL	o	+	±	o	o	o	+	+	o	Subarachnoid hemorrhage	Tr. pr., 1:1,280
F.W.	F	34	32	+	o	o	15	150–28,500	170/130	+P	+	+	+	+	o	+	+	+	o	Cerebral hemorrhage	BUN, 62 Tr. pr., 1:1,280
A.K.	M	44	40	o	o	o	3	5,600	125/85	+P	+	+	o	o	o	o	o	+	o	? (No head examination)	
H.P.	M	63	58	o	o	+	6	2,500–13,000	130/90	o	+	o	+	+	o	+	+	+	+	Cerebral hemorrhage	
E.G.	F	21	17	+	o	+	20	2,100–11,000	150/110	+PL	+Fl	+	±	+	+	o	+	o	+	Cardiac failure	
R.L.	F	*	45	+	o	+	*	0–8,400	120/80	o	+	+	±	+	+	+	+	o	+		Tr. pr., 1:1,280
R.E.	M	†	9	o	o	+	†	130–28,400	170/120	+P	+	+	+	+	+	+	+	o	o		Positive blood culture (see text, p. 278) Tr. pr., 1:1,280

M = male, F = female; + = present or positive, ± = doubtful, O = absent or negative; P = pneumonia, PL = Loeffler's pneumonia, Fl = failure; BUN = blood urea nitrogen; Tr. pr. = serum precipitin test with trichinella antigen.

* Last seen in April, 1950, at the age of 56. Duration of illness at that time, 66 months.

† Last seen in April, 1950, at the age of 12. Duration of illness at that time, 20 months.

ures. In individual cases the eosinophil count fluctuated markedly during the course of the illness (Text-fig. 1). Occasionally it followed a peaked curve with a rapid drop shortly before death. In some cases it was persistently high, while in others it fluctuated between very high and normal. Such findings stress the importance of repeated differential blood counts. As in any wasting disease, anemia and weight loss were common accompaniments, the latter often being very marked, and out of proportion to the degree of fever.


Eosinophil count (in thousands of cells per cmm. of blood)
X CASE M.M.
X CASE F.W.
NORMAL RANGE
X CASE G.D.
MONTHS
X-EXPIRED; <·····> DENOTES LAPSE OF SEVERAL MONTHS

Text-figure 1. Behavior of eosinophil count in three cases of allergic granulomatosis.

Recurrent episodes of pneumonia were present in nearly every case. Five presented the typical clinical and roentgenologic findings of Loeffler's pulmonary infiltration. Association of Loeffler's pneumonia with asthma and periarteritis nodosa has been reported (Hennell and Sussman [14]). Radiology 1945, 44:324

Numerous symptoms indicated involvement of various organ systems. Elevation of blood pressure was noted in 7 cases, particularly in those of longer duration, usually appearing in the latter part of the illness. Heart failure due to myocardial damage was frequent. Abdominal pain and diarrhea, often bloody, were present in nearly every

case. As a rule, there was mild hematuria and albuminuria; nitrogen retention occurred in only 3 cases. The majority of patients exhibited a variety of cutaneous manifestations, erythematous, maculopapular, or pustular. Of significance is the frequency of purpura (non-thrombocytopenic). The other lesion that merited special attention was a deep cutaneous or subcutaneous nodule, occurring on the head, trunk, and extremities. Such nodules were encountered in 7 cases; in 5 of these they furnished the anatomical basis (by biopsy) for the diagnosis of allergic granuloma. Generalized, though mild, lymphadenopathy was observed in over one-half of the cases. Manifestations of involvement of the central and peripheral nervous systems were very common. The former varied from disorientation to convulsions and coma; 3 of the patients died of cerebrovascular accidents. Peripheral neuropathy with variable motor, sensory, and trophic disturbances of the extremities and of the cranial nerves was observed in 8 of 13 cases.

Symptoms referable to joint involvement were not common: in 3 instances they were mild; in another they were severe enough to warrant the diagnosis of rheumatoid arthritis.

Of interest was the occurrence of a positive precipitin test with trichinella antigen in all 4 patients on whom the test was performed. Although the precipitin titer was very high (1:1, 280), the skin tests with trichinella antigen were negative. Similar findings have been reported in periarteritis nodosa.[15]

A summary of the clinical data is found in Table I.

Anatomical Findings

Because of the uniformity of findings, only a brief summary of the gross observations is presented. In the majority of cases the gross findings were those of periarteritis nodosa. There were nodular swellings along the course of small arteries of many organs, including the heart, liver, spleen, kidneys, gastro-intestinal tract, gallbladder, pancreas, and others. These vascular changes were accompanied by infarcts, hemorrhage, and scars in the affected organs. In a number of cases there was grossly recognizable vascular involvement of the lungs, consisting of thickening of branches of the pulmonary arteries and narrowing of their lumina, and occasional occlusion by a thrombus. In several instances there was patchy pneumonic consolidation, mostly confined to the lower lobes. In 4 cases the bronchi were filled with tenacious mucus.

Of all the organs, the heart was most prominently affected. Five cases showed pericarditis, 2 of them in an acute fibrinous stage. The heart usually was enlarged, both ventricles being involved. In addition

to the vascular changes mentioned, there were varying degrees of patchy myocardial scarring, and, in some cases, endocardial fibrosis accompanied by adherent mural thrombi, sometimes present in all four chambers. In no instance were there any valvular changes suggestive of rheumatic disease.

In 2 instances additional changes, which merit a more detailed description, were encountered in various organs.

Case A. K.* was characterized by the presence of widespread nodules, similar in appearance to those seen in miliary tuberculosis. They were well demarcated, firm, and glistening white or yellow, measuring up to 2 mm. in diameter. The lungs were thickly studded with these nodules and markedly increased in volume and weight (nearly 3,000 gm.). The spleen was considerably enlarged, weighing 460 gm., and showed innumerable nodules up to 1 cm. in diameter (Fig. 1). The same nodules were seen in the liver and in both kidneys, where they often coalesced to form larger masses.

Miliary nodules and larger aggregates were found also in the spleen of case H. P. This spleen was slightly enlarged (190 gm.) and was riddled with innumerable grayish, sometimes confluent nodules, averaging 1 to 2 mm. in diameter (Fig. 2). In this case there were no gross changes in the arteries, apart from arteriosclerosis of large vessels. However, the portal vein, just beyond the bifurcation, presented a raised, brownish plaque, about 2 cm. in diameter, which appeared to involve the entire thickness of the wall. In the heart, in addition to the gray streaks through the myocardium, there was marked thickening of the endocardium, particularly at the apex of the left ventricle, caused by deposition of gray, semi-translucent tissue under the smooth endothelial lining. The kidneys contained many cysts, probably on an arteriosclerotic basis. The parenchymal markings were somewhat obscured. The pelves and the ureters had unusually thick and rigid walls, and their mucosa was brownish gray and nodular. The ureters were slightly dilated.

Microscopic Changes

The histologic changes in all 13 cases were essentially similar except for minor variations. They are, therefore, described together in order to avoid repetition.

Connective Tissue

The important feature in nearly all cases (12 of 13), were lesions of extravascular connective tissues, including the stroma of various organs. Such lesions were, on occasion, very widespread, but more often they were found in just a few sites, or even in only one (Table II).

* The autopsy in this case was performed by Dr. Peter Gruenwald.

TABLE II
Anatomical Findings in 13 Cases of Allergic Granulomatosis Location of Principal Extravascular and Vascular Lesions

TABLE II

Anatomical Findings in 13 Cases of Allergic Granulomatosis
Location of Principal Extravascular and Vascular Lesions

Case	Extravascular lesions	Arterial lesions			Venous lesions
		Active	Healed	Granulomatous	
S.W.	Epicardium	Lungs, heart, kidneys, gastro-intestinal tract	Lungs, liver, gastro-intestinal tract	Kidneys, gastro-intestinal tract	
C.D.	Epicardium	Heart, liver, spleen, pancreas, kidneys, uterus, gallbladder, gastro-intestinal tract	Lungs, liver, pancreas, kidneys, gastro-intestinal tract	Heart, pancreas, kidneys, gallbladder, gastro-intestinal tract	
S.S.	Epicardium, perjaortic tissue, liver, skin (biopsy)	Lungs, gastro-intestinal tract	Heart, liver, spleen, gallbladder, gastro-intestinal tract	Lungs, gastro-intestinal tract	Gastro-intestinal tract
B.Z.	Skin (biopsy)	Heart, spleen, kidneys, (healing)	Heart, liver, kidneys, gastro-intestinal tract		
F.H.	Skin (biopsy)	Uterus (healing)	Lungs, liver, kidneys, uterus, gastro-intestinal tract		
H.C.	Epicardium				
M.M.	Epicardium, myocardium, lungs	Heart, liver, kidneys, pancreas, diaphragm, gallbladder, gastro-intestinal tract	Heart, liver, spleen, pancreas, kidneys, gastro-intestinal tract	Heart, liver, kidneys, gallbladder	Heart (myocardial veins)
F.W.		Liver, kidneys, gallbladder, nerves, gastro-intestinal tract, adrenals, muscles	Lungs, heart, liver, adrenals, nerves, gastro-intestinal tract, muscles	Gastro-intestinal tract	Heart (large coronary vein)
A.K.	Lungs, spleen, liver, kidneys	Lungs, spleen, liver, kidneys, gallbladder		Lungs, liver, gallbladder, kidneys	Lungs (pulmonary veins), spleen (trabecular veins)
H.P.	Epicardium, myocardium, endocardium, spleen, kidneys, perirenal fat, ureters	Spleen (trabecular and follicular arteries), ureters	Ureters, adrenals	Spleen (trabecular arteries), ureters	Liver (portal vein), spleen (trabecular veins)
E.G.	Skin (biopsy)	Unknown			Unknown
R.L.	Skin (biopsy)	Gallbladder (biopsy)	Unknown		Unknown
R.E.	Skin (biopsy)	Unknown			Unknown

Inflammatory changes were always present, varying in intensity from diffuse sheets of exudate (Fig. 3), to small localized collections of inflammatory cells. The composition, as well as the extent, of the exudate seemed to depend on the phase of the inflammation, whether acute, subacute, or chronic. In the acute stage the predominant cell was the eosinophilic leukocyte which constituted as much as 70 to 80 per cent of the exudate. The number of eosinophils decreased as the acute inflammation subsided, but they were seldom entirely absent. Further characteristic elements were macrophages and giant cells, either of foreign body or of Langhans' type; these became more prominent as the process tended toward the subacute and chronic stages. In addition, there were varying numbers of plasma cells, lymphocytes, and neutrophils.

The most frequent site of inflammation was the heart, and, more particularly, the epicardium; but its manifestations were observed, as well, in the lungs, bile ducts, spleen, kidneys, ureters, perirenal fat, lymph nodes, muscles—in short, in any part of the body.

The most characteristic extravascular lesion was a granulomatous nodule (Figs. 4 and 5) associated with, or replacing, the inflammatory exudate and often located near a small vein. These nodules ranged in size from about 50 μ to 1 mm. or more, the latter being visible to the naked eye. They showed a central, eosinophilic core surrounded by radially arranged macrophages and giant cells. The eosinophilic core consisted of two elements: necrotic cells and severely altered collagen fibers. These two elements were not always clearly discernible at the same time, and not necessarily present in every instance. The cells involved in the process of necrosis were predominantly eosinophilic leukocytes, and, to a lesser extent, macrophages and local tissue cells. Small, dense aggregates of eosinophils occurred within the exudate in the acute stage. The leukocytes were at first intact, but subsequently underwent disintegration, with scattering of the eosinophilic granules and pyknosis and fragmentation of the nuclei (Fig. 6). The macrophages around such a focus assumed the appearance and the radial arrangement of epithelioid cells, although often their cytoplasm was less abundant and the nuclei more shrunken and distorted than those of true epithelioid cells. With Mayer's hematoxylin stain the fragmented nuclei frequently gave the necrotic center of the nodule a bluish cast; however, occasionally this was due to deposition of calcium salts. Apparently the cellular débris was removed, as in the majority of the nodules one could see only fragmented, granular, eosinophilic material without a trace of the pre-existing cellular elements. In some foci, epi-

thelioid cells predominated among the cellular elements in and around the area of necrosis, while giant cells were inconspicuous or entirely absent. In such cases the necrotic focus occupied the center of a nodule of epithelioid cells, and, as the lesion spread peripherally, additional layers of cells became necrotic. Such structures sometimes resembled epithelioid tubercles.

The second structural element of the granulomatous nodule consisted of severely altered collagen fibers. In the acute stage, collagen changes often were overshadowed by necrotic inflammatory cells, unless studied by connective tissue stains. With these stains it was possible to demonstrate an increasing acidophilia of the thin fibers, followed by complete loss of tingibility and eventual total disappearance. Fiber changes were best seen in lesions in which cellular exudate was negligible, particularly in the more chronic cases. The collagen change was of the nature of so-called fibrinoid swelling. The fibers became very thick, straight, and developed increased affinity for acid dyes. Along the edges of such fibers, splintering of short, thin fragments was observed; these were somewhat wavy, "spirochete"-like, or rod-like, and tended to break up into highly acidophilic granular masses. With special stains, such as the modified Mallory stain, the altered fibers showed transitions from blue-green to yellow. In polarized light, the fibers exhibited complete loss of the normal birefringence.

One might expect that much of the inflammatory exudate would undergo resorption; however, the more severe alterations in the tissues, particularly those leading to necrosis, apparently resulted in scarring with proliferation of fibrous tissue. The final scar did not differ from scar tissue in general, except when there were remnants of specific inflammation or necrotic foci. Scarring was most prominent around the blood vessels, and in the heart where it occurred in all three layers. In the epicardium, there was replacement of fat and fine reticular fibers by thick collagen bundles; in the myocardium, interstitial fibrosis extending from the septa indicated, at least in part, preceding interstitial inflammation; the endocardial scars often were accompanied by mural thrombosis. On occasion, secondary collagen change occurred in the scar tissue, usually accompanied by typical granulomatous proliferation.

Blood Vessels

Lesions of blood vessels were present in 9 of 10 autopsied cases, although on occasion, as in case H. P., they were few and inconspicuous. In the tenth case (H. C.), there were only a few scarred arterial lesions suggestive of arteriosclerosis rather than of a previous inflammation

and necrosis. The arterial alterations were essentially similar to those usually seen in periarteritis nodosa, as far as the character of lesions and organ distribution were concerned (Table II). At the same time they were related to the changes in the extravascular connective tissue described in the preceding paragraphs. All stages of necrotizing arteritis could be seen in nearly every case, although the healed, fibrosed lesions usually predominated. This is not surprising, in view of the long clinical course in the majority of cases. In the acute phase one could observe typical segmental "fibrinoid" necrosis of vessel wall, with marked swelling, destruction of the muscle and elastic tissue, and often, formation of an aneurysm. This was accompanied by an inflammatory response in and around the vessel, with predominance of eosinophilic leukocytes. The lumen often was blocked by a thrombus or by markedly proliferated endothelial cells, as emphasized by Bergstrand.[11] Narrowing of the lumen also frequently resulted from the reparative fibrosis and scarring of the affected wall. The final appearance of a healed lesion was indistinguishable from the end stage of any type of arteritis.

An important feature in many arterial lesions was the presence of macrophages and giant cells around necrotic areas, the macrophages frequently assuming the radial arrangement and appearance of epithelioid cells (Figs. 7, 8, and 9). This granulomatous response was found in at least some of the arteries in 7 of 9 cases showing active or healing lesions (Table II). In 2 instances (C. D., S. S.) many of the macrophages in the arterial media had vacuolated, foamy cytoplasm, as if loaded with lipids.

Alterations of the veins were less frequent and less prominent than those of the arteries. They were encountered in 5 cases (Table II). They bore considerable resemblance to the lesions in the extravascular connective tissue, rather than to those in arteries, consisting of acute inflammatory foci within the wall, usually accompanied by typical granulomata (Figs. 3 and 10). In some of these, healing was followed by formation of a scar.

Although the histologic appearance of the lesions described was rather uniform throughout the body, there were some peculiarities noted in individual organs, which merit special consideration. Among these were the heart, lungs, and kidneys, as well as the liver, spleen, lymph nodes, and skin.

Heart

Of all the organs in the body, the epicardium was the most common seat of granulomatous nodules (Fig. 5; 6 of 10 autopsied cases, Table II). The nodules were, as a rule, accompanied by inflammation and fibrosis, as previously described. The most constant lesion of the myo-

cardium was interstitial eosinophilic inflammation; this was present in 7 cases, varying in extent from an occasional focus to diffuse myocarditis. Granulomatous nodules, when they occurred, usually were situated in the connective tissue septa, on occasion closely simulating the myocardial Aschoff body of rheumatic fever. As previously mentioned, fibrosis of the myocardium was common; it probably resulted from anoxia secondary to vascular lesions, as well as from scarring of inflammatory foci. Valvular lesions usually were absent, but often the mural endocardium was involved, probably by extension from the myocardium. This resulted in patchy and diffuse subendocardial inflammation followed by fibrosis. In one case (H. P.) a small granuloma was found on a chorda tendinea of the mitral valve.

Lungs

Involvement of branches of the pulmonary artery was common (6 of 9 cases; Fig. 9). The veins were involved in one case (Fig. 10, Table II). In approximately one-half of the cases there were parenchymatous lesions in the form of a more or less extensive pneumonic process, involving septa as well as the alveoli (Figs. 11 and 12). In the acute stage the exudate was characterized by predominance of eosinophilic leukocytes, mixed with giant cells. The latter were very numerous in case M. M. Frequently, healing terminated in focal fibrosis (case H. P.). In 2 cases (M. M., A. K.) there were typical granulomatous nodules in the septa. These findings are very similar to those reported in Loeffler's pneumonia,[16,17] as already noted by several authors.[11,18,19]

Histologic evidence of bronchial asthma (hyalinization of basement membrane, increased mucous secretion, eosinophilic infiltration of the bronchial walls) was present in most of the cases, but was never very marked.

Kidneys

Acute or chronic renal vascular lesions and their sequelae were common. Frequently there was diffuse or focal interstitial nephritis with predominance of eosinophilic leukocytes. In some cases, this was accompanied by granulomatous nodules. In one instance (H. P.), the inflammation extended into the peripelvic fat and involved the entire length of both ureters. Most of the cases showed focal glomerular lesions (Fig. 13), usually involving only one or a few capillary loops, and rarely affecting the majority of glomeruli. Usually only the terminal stage, that is, fibrosis with complete obliteration of the involved loops and capsular adhesions, was seen. These changes bore considerable similarity to healed Löhlein's lesions. However, in 2 cases (M. M., H. P.) one could observe also acute lesions which consisted of marked

swelling and intense eosinophilia of the capillary walls, with necrosis of cells and fragmentation of the nuclei. Frequently the afferent arterioles and the pre-arterioles were involved in the same process, so that the fibrinoid change could be traced from an arcuate artery to the glomerular capillaries.

Tubular lesions were not constant and were mostly non-specific. In several instances many tubules contained granular material resulting from disintegration of eosinophilic leukocytes and lining cells of the tubules.

Liver

Lesions in the liver usually were confined to the blood vessels with resulting damage to the parenchyma. An occasional case (A. K.) showed extravascular granulomatous nodules. Of special interest in one case (S. S.) was affection of a large bile duct. This consisted of necrosis of the wall, infiltration by bile pigment, marked inflammation with exudation of eosinophils and plasma cells, and, finally, proliferation of fibroblasts.

Spleen

In the majority of cases there were no specific lesions in the spleen other than those of hilar or trabecular arteries. In one instance (H. P.) the follicular arteries showed prominent fibrinoid and hyaline changes, and in 2 cases there was inflammation of the trabecular veins (H. P., A. K.). In the same 2 cases, the spleen was the seat of extensive and striking parenchymatous changes. As mentioned, the gross appearance was suggestive of miliary tuberculosis; it was caused by discrete and conglomerate granulomatous nodules in various stages of development (Fig. 4). Many of these nodules were scattered at random throughout the red pulp; others lay in close relation to the trabeculae, encroaching upon the latter. The collagen fibers were spread apart by the infiltrating cells, sometimes appearing swollen and eosinophilic, sometimes thin and pale. Many of the fibers disappeared, with marked attenuation of the trabeculae. Healing of the nodules occurred by the usual process of fibrosis (case H. P.).

Lymph Nodes

Because of frequent generalized lymphadenopathy, many lymph nodes were removed at autopsy for histologic examination. Usually they showed non-specific, although sometimes quite extensive, inflammatory changes. In one case (H. P.), a lymph node removed for biopsy 5 months before death, at the height of the acute illness, revealed diffuse granulomatous inflammation with obliteration of architecture, proliferation of reticulum cells, eosinophilic infiltration, and an occasional

giant cell of Langhans' type. This picture was suggestive of early Hodgkin's disease. However, at autopsy, numerous lymph nodes showed only chronic inflammation with predominance of plasma cells.

Skin

Sections of cutaneous nodules were available in 6 cases. These nodules were located just beneath or within the dermis, and showed conglomerate and confluent granulomatous lesions with necrosis of collagen, and epithelioid, giant cell, and eosinophilic reaction (Fig. 14). The location of the nodules was sometimes paravascular; but, in no instance were lesions observed in the vessel walls.

In 3 cases, the granulomata were located in the subcutaneous tissue, and were identical with the nodules seen in the viscera (S. S., F. H., R. E.). In 2 others (E. G., B. Z.) the nodules were situated in the dermis and were less distinctly circumscribed (Figs. 15 and 16). They were characterized predominantly by severe fibrinoid alteration of collagen. In the sixth case (R. L.) there was homogenization of collagen in the lesion, similar to that seen in the nodules of rheumatoid arthritis. In this case there were severe joint symptoms.

Nerves

Sections of peripheral nerves were available in only one case (F. W.). These showed acute inflammation and necrosis of the walls of the nutrient arteries.

Comment

As mentioned above, of 14 cases exhibiting asthma, fever, and hypereosinophilia, 13 presented characteristic lesions. These consisted of infiltration of connective tissue with eosinophilic leukocytes, and fibrinoid change of collagen with granulomatous (epithelioid and giant cell) reaction in connective tissue and in the blood vessel walls. The remaining case * showed at the time of necropsy only completely fibrosed scars in the walls of many arteries and scars in the retroperitoneal connective tissue and the pericardium. In cases B. Z. and F. H. there were only a few subsiding lesions at the time of death in addition to the numerous vascular scars, and the diagnosis of allergic granulomatosis rests on biopsy of cutaneous nodules. This emphasizes the diagnostic importance of these nodules.

The presence of giant cells around necrotic collagen in the blood vessel wall has been noted by some of the early observers in reporting cases

* This case as well as cases S. S. and F. H. have been reported previously in detail by Harkavy.[5]

of periarteritis nodosa associated with asthma (Otani [20]). Similarly, giant cell reaction and fibrinoid alteration of extravascular collagen have been reported in cases of periarteritis nodosa ascribed to drug allergy (iodine,[7] dilantin [8]). There are cases on record in which giant and epithelioid cell reaction was found in the absence of allergic history,[18,21] but in at least some of them the author (Ophüls [21]) admits that the history was incomplete and that the anatomical changes in the lungs were strongly suggestive of asthma. Clearly, review of the literature cannot be relied upon to determine the relative frequency of granulomatous reaction and of extravascular lesions of collagen in cases with allergic background and those without such a background, because the clinical stories and, particularly, the microscopic descriptions are very often presented in an abbreviated form. At least a partial answer can be obtained only by comparison of available cases.

As might be expected, most of the cases reported in this series were indexed under the heading of periarteritis nodosa in the Pathology Laboratory of the Mount Sinai Hospital. Under the same heading there were 20 odd cases with no history of allergy, particularly none of asthma. We reviewed the clinical records and the pathologic material of all cases, numbering 15, in which histologic slides were available. Only one of these showed fleeting eosinophilia up to 1,300 cells per cmm. (14 per cent of 9,400 white cells); a few had an occasional eosinophil count of between 400 and 1,000, while in all other cases eosinophils were below 400, or absent. These findings are in agreement with those of Wilson and Alexander [8] who, in their review of 300 published cases of periarteritis nodosa, found that high eosinophilia was present in 94 per cent of cases associated with asthma and in only 6 per cent of those without a history of asthma.

The histologic slides in these 15 cases were searched for evidence of extravascular alteration of collagen and for epithelioid and giant cell reaction around altered collagen, whether extravascular or within the vessel wall. Extravascular changes were observed in 2 instances. In one, there were definite foci of fibrinoid alteration in the posterior mitral leaflet, accompanied by formation of ridge-like projections on the auricular surface of the valve. Less advanced collagen changes were found in the fibrous septa of the myocardium, usually near small blood vessels; these lesions were surrounded by myocytes and Aschoff cells, and were closely reminiscent of rheumatic nodules. In the second case, small foci of severe collagen alteration, apparently not connected with blood vessels, were seen in the splenic trabeculae. In the remaining cases of this group there were no extravascular lesions, and in none of

the 15 cases could we find epithelioid or giant cells around the altered collagen.

Recently, Zeek, Smith, and Weeter [22] described the morphologic lesions of what they called "hypersensitivity angiitis." Included in this group were patients who developed reaction to various drugs, particularly sulfonamides, and also one case of severe asthma. At autopsy they exhibited necrotizing arterial lesions in many organs, and, in addition, frequent involvement of veins, pulmonary vessels, splenic trabeculae and follicular arterioles, and necrotizing lesions in the renal glomeruli. In addition, there occurred edema of the interstitial tissue of the viscera and small foci of necrosis, and, occasionally, the interstitial tissue was infiltrated with inflammatory cells of various types, including eosinophils. As can be seen, these lesions are very similar to those observed in our series, although Zeek made no mention of epithelioid and giant cell reaction except to state that foreign body giant cells never occurred in experimental "true" periarteritis nodosa.

It has been the contention of many authors (Otani,[20] Zeek *et al.*,[22] Mallory [23]) that what is called periarteritis nodosa is not a single entity, but a heterogenous group of cases whose common denominator is the necrotizing arterial lesion. Zeek believed that there are at least two distinct types: the above-mentioned hypersensitivity angiitis and the "true" periarteritis nodosa. It seems to us that there are valid reasons, both clinical and anatomical, for defining the group here presented under the name of allergic angiitis and allergic granulomatosis (Churg and Strauss [24]). Relation of this group to the hypersensitivity angiitis of Zeek remains to be established. Its allergic etiology seems to be clearly proved, at least as long as asthma is considered an allergic disease. This does not mean that other cases classified as periarteritis nodosa may not also have an allergic etiology. However, if one excludes cases associated with asthma, and those due to drug and serum sickness, all of which constitute probably less than one-third of the entire group of periarteritis nodosa, there is very little clinical evidence of sensitization in the remaining two-thirds of the cases. It is true that arterial lesions very similar to those of periarteritis nodosa have been produced by injection of foreign serum in animals. We have no personal experience with these lesions. It seems to us, however, that it would be important to determine whether these vascular alterations truly reproduce periarteritis nodosa or, with due regard to species difference and the mode of sensitization, allergic or hypersensitivity angiitis.

In the cases here presented, allergic granuloma occurred in combination with angiitis, except for one instance in which vascular involvement

could not be demonstrated. There is no doubt that eosinophilic and granulomatous inflammation can exist quite independently of vascular involvement. These lesions have been observed in the lungs of patients with Loeffler's "pulmonary infiltration with eosinophilia,"[16,17] in the livers of children exhibiting marked eosinophilia (Zuelzer and Apt[25]), and in the hearts of individuals who developed allergic reaction to arsphenamides (Šikl[26]). The allergic nature of Loeffler's syndrome is generally accepted. In the cases described by Zuelzer and Apt there also is considerable evidence for the allergic mechanism giving rise to the granulomata in the liver. The occurrence of similar lesions in the appendiceal wall invaded by parasites, such as ascaris and strongyloides, gives support to this concept (Wenger[27]). It seems that when the anatomical involvement spares the vascular system, the clinical course is likely to be much milder and often self-limited, while the majority of cases with widespread vascular lesions end fatally.

Little can be said about the morphogenesis of allergic granulomatosis. Presumably, as a result of sensitization to inhalants, bacteria, or drugs, the antigen-antibody reaction produces or liberates a chemical substance or substances which damage collagen fibers and attract eosinophilic leukocytes. Whether collagen damage always precedes inflammatory exudation cannot be stated with certainty, although it often appears to do so. Unfortunately, we know nothing about substances that attract eosinophils. Although the altered collagen appears as in other types of periarteritis nodosa, there must be a difference in its chemical composition, because it excites epithelioid and, particularly, giant cell reaction. This difference may be due to substances responsible for the original damage to the collagen, or perhaps to some factor contributed by the eosinophilic leukocytes. In favor of the latter concept is the occurrence of giant cells in association with eosinophilic pneumonia, but in the absence of obvious collagen changes. Also, somewhat similar necrotic and granulomatous lesions were observed in certain cases of Hodgkin's disease, in lymph nodes rich in eosinophils.

Summary and Conclusions

The occurrence of a clinical syndrome of severe asthma, fever, and hypereosinophilia, together with symptoms of vascular embarrassment in various organ systems, has been established. Thirteen observed cases of this syndrome formed the basis of this study.

The basic anatomical changes in these 13 cases consisted of widespread vascular lesions of the type seen in periarteritis nodosa, and of characteristic tissue alteration in the vessel wall and in the extravascular collagen system.

This tissue alteration, common to all cases here reported, comprised necrosis of eosinophilic exudate, severe "fibrinoid" collagen change, and granulomatous proliferation of epithelioid and giant cells. This is considered a histopathologic entity termed "allergic granuloma."

The finding of the granulomatous lesions, both within vessel walls and in connective tissue throughout the body, suggests that this syndrome constitutes an entity apart from classical periarteritis nodosa. This assumption was corroborated by review of 15 cases of periarteritis nodosa without asthma, none of which revealed extravascular granulomata or granulomatous vascular changes.

Of frequent occurrence in this syndrome are cutaneous and subcutaneous nodules showing the typical connective tissue alteration. These are of significant value for diagnosis by biopsy. Of further possible diagnostic significance is granulomatous lymphadenitis.

It is suggested that other allergic syndromes (Loeffler, Zuelzer, Šikl) may represent the more benign forms of allergic granulomatosis, while angiitis is its most malignant expression.

ADDENDUM

As of January, 1951, patients R. L. and R. E. were living at the ages of 57 and 13 years, respectively. Duration of illness at that time was 73 and 29 months, respectively.

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DESCRIPTION OF PLATES

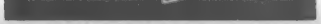
PLATE 48

FIG. 1. Case A. K. Gross appearance of the spleen. Subcapsular and parenchymal nodules.

FIG. 2. Case H. P. Section of spleen. Discrete and confluent nodules in the pulp.

FIG. 3. Case H. P. Portal vein. Diffuse inflammatory infiltration with predominance of eosinophils, and scattered granulomata within the exudate. × 55.

FIG. 4. Case H. P. Spleen. Typical granuloma showing necrotic center surrounded by radially arranged epithelioid cells and a few giant cells. × 295.



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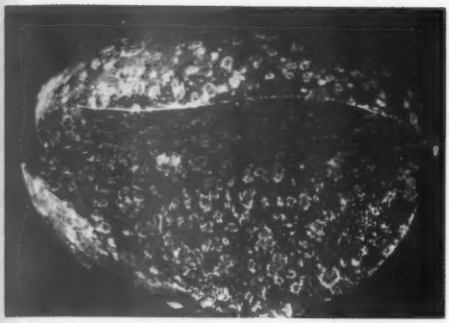

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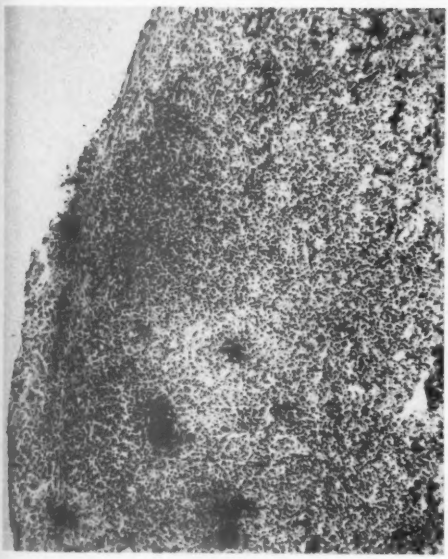

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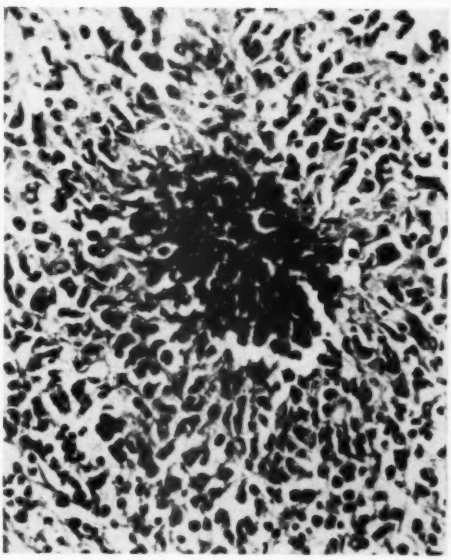


4

Churg and Strauss

Allergic Granulomatosis and Vascular Disease

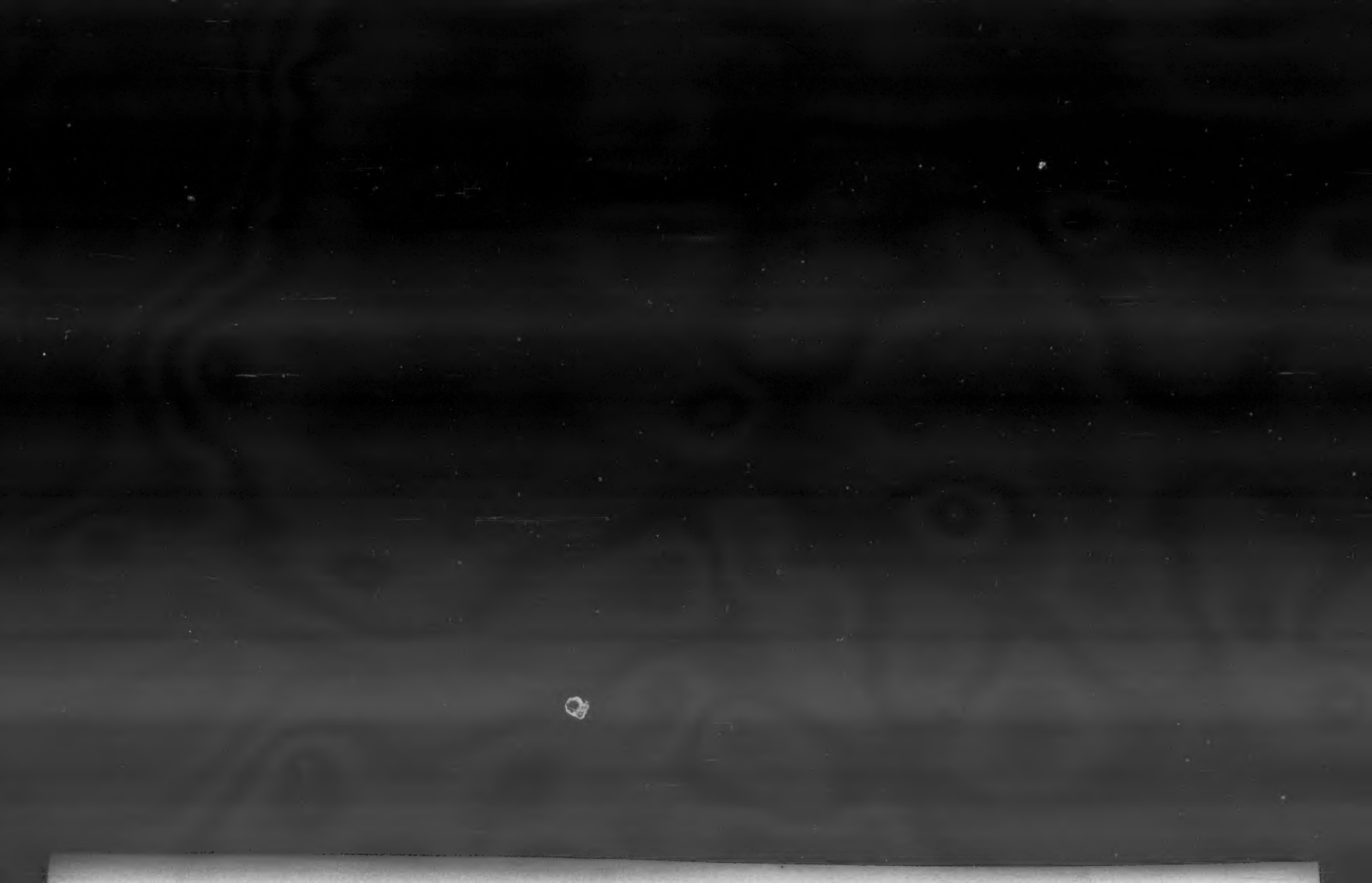
PLATE 49

FIG. 5. Case H. C. Epicardium. Granuloma with numerous giant cells. × 200.

FIG. 6. Case H. P. Inflammatory exudate showing focus of clumping and disintegration of eosinophils. × 525.

FIG. 7. Case M. M. Coronary artery. Subintimal granulomata around necrotic collagen. × 30.

FIG. 8. Same as Figure 7, at a higher magnification. × 200.



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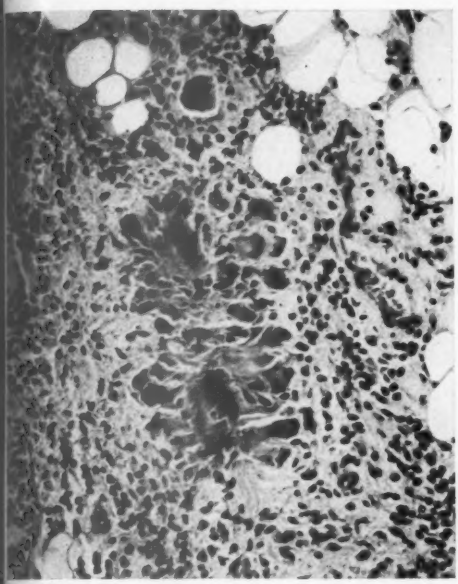

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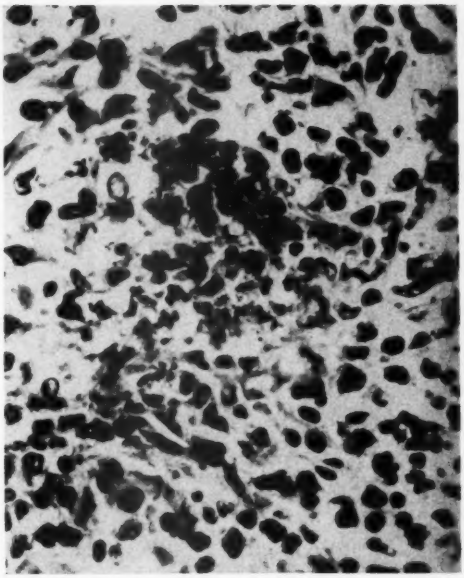

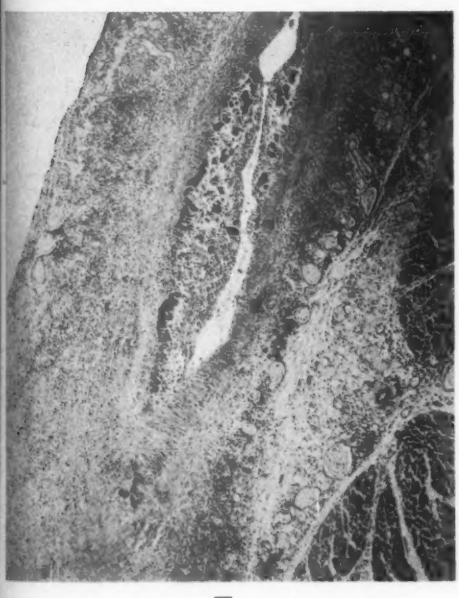

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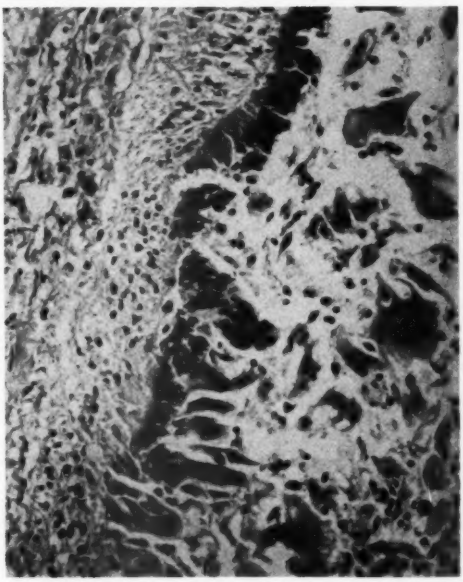

8

Churg and Strauss

Allergic Granulomatosis and Vascular Disease

PLATE 50

FIG. 9. Case S. S. Lung. Branch of a pulmonary artery showing marked narrowing of the lumen and a granuloma in the wall. × 100.

FIG. 10. Case A. K. Lung. Branch of a pulmonary vein showing a large subintimal granuloma. × 100.

FIG. 11. Case M. M. Lung. Eosinophilic pneumonia with necrosis of exudate, and giant cells. × 100.

FIG. 12. Same as Figure 11, at a higher magnification. Giant cells, edema, and infiltration of alveolar septa with eosinophils. × 375.

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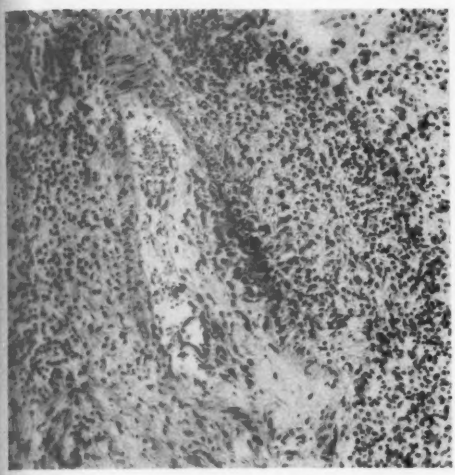

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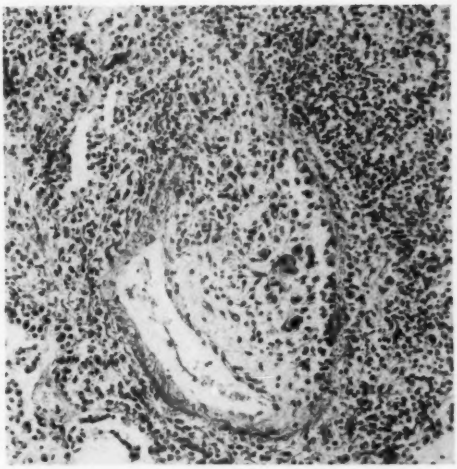

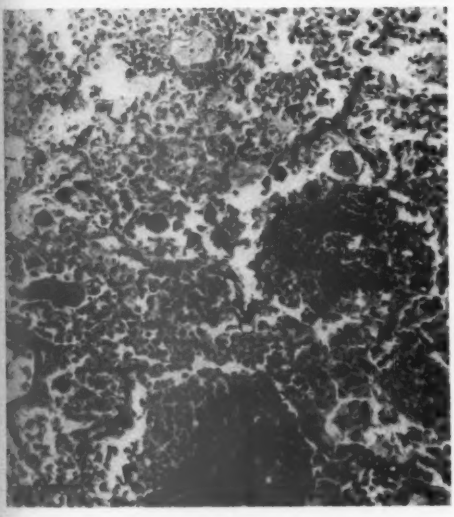

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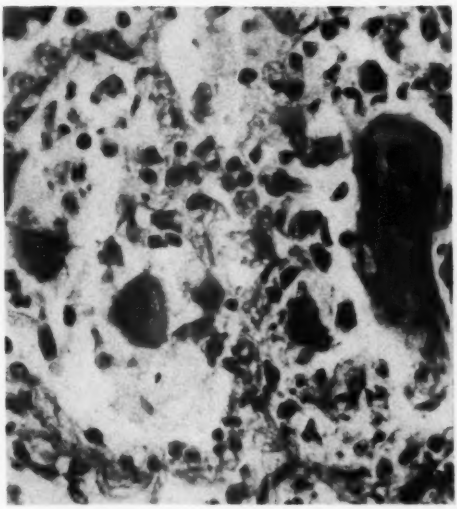

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Churg and Strauss

Allergic Granulomatosis and Vascular Disease

PLATE 51

FIG. 13. Case M. M. Kidney. Focal necrotizing glomerulitis. × 330.

FIG. 14. Case S. S. Subcutaneous tissue. Diffuse inflammatory exudate and conglomerate granulomata. × 35.

FIG. 15. Case B. Z. Skin. Dermal granulomatous nodule with marked collagen change. × 60.

FIG. 16. Same as Figure 15, at a higher magnification. Junction of necrotic collagen with epithelioid cell zone. Many polymorphonuclear leukocytes may be noted. × 415.

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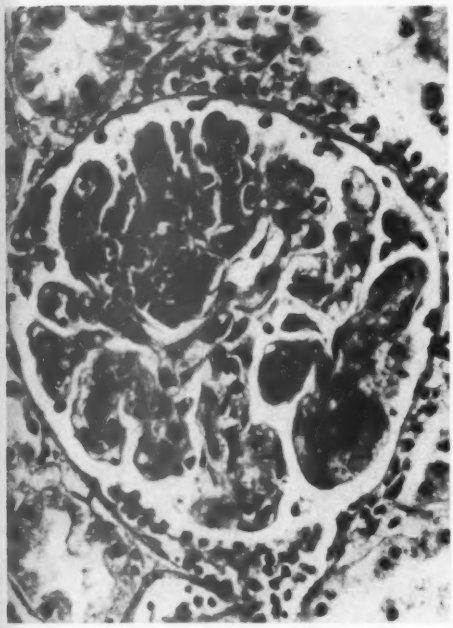

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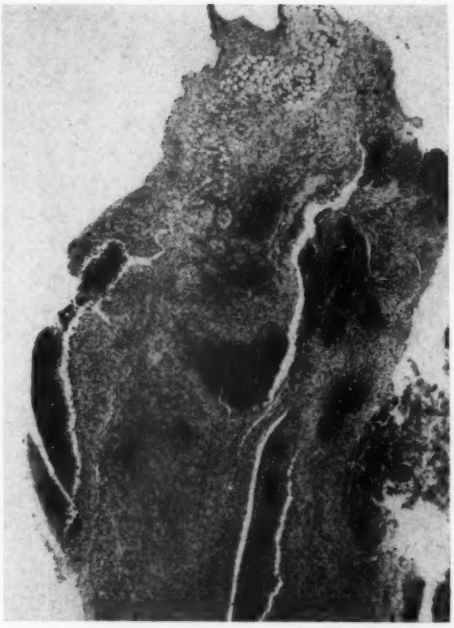

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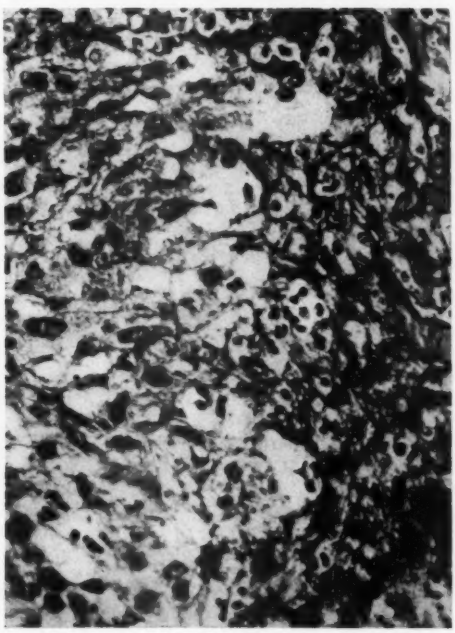

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Allergic Granulomatosis and Vascular Disease

POST-TRAUMATIC SUBCUTANEOUS GRANULOMAS ASSOCIATED WITH A CRYSTALLINE MATERIAL *

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The purpose of this paper is to report 7 cases in which granulomatous lesions were found associated with traumatically introduced crystalline material. In all of these cases there was a definite history of trauma with laceration of the skin, healing *per primam,* a latent interval of 10 to 35 years, then the development of a subcutaneous nodule. Microscopically, all of these lesions were characterized by non-caseating tubercles, anisotropic crystalline material, and giant cells of the foreign body type. In most of these cases, according to the history, the foreign material contained silica. These lesions probably represent traumatic silicosis of the skin. The diagnosis is difficult, since a history of trauma may not be elicited. Also, these lesions may be readily mistaken for Boeck's sarcoid to which they have a strikingly similar histologic appearance.

Similar cases of granulomas of the skin have been described by King,[1] who reported "sarcoid-like" lesions, secondary to trauma. Three of his patients had been cut by glass, and in a fourth case the foreign material was considered silica. German [2] has described a "lupoid-sarcoid" lesion in the skin and subcutaneous tissue of the scalp, which was associated with crystalline material and occurred many years following the initial laceration. He believed this crystalline material to be silica. Grier, Nash, and Freiman [3] have described subcutaneous granulomas, following laceration of the skin by fragments of broken fluorescent lamp bulbs. They considered the granulomatous tissue response to be due to zinc manganese beryllium silicate which is contained in the fluorescent lamp bulbs.

In this paper, in addition to presenting the case reports, we shall discuss the spectroscopic analysis of the tissue, the differential diagnosis between these lesions and Boeck's sarcoid, the staining reactions of the lesions, and studies with the polarizing microscope.

REPORT OF CASES

The essential data on these cases are shown in Table I.

* The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

Received for publication, May 31, 1950.

TABLE I

Summary of Cases

Case	Age	Race	Sex	Latent period	Duration	Material producing trauma	Site of lesion	Roentgenologic findings	Original pathologic diagnosis	Crystallography
[1] (NMS 48–4471)	27 yrs.	W	F	15 yrs.	2 wks.	Coal	Forehead	Not done	Boeck's sarcoid	Anisotropic crystals and amorphous anthracite
[2] (NMS 24564)	28 yrs.	W	F	15 yrs.	6 wks.	Slate	Forehead	Bone erosion present	Boeck's sarcoid	Anisotropic crystals
[3] (NMS 48–4421) (NMS 49–521)	27 yrs.	W	F	17 yrs.	1 mo.	"Dirty wound" (struck forehead on tree)	Forehead	Negative	Silicosis, skin	Anisotropic crystals
[4] (NMS 48–3639)	27 yrs.	W	M	12 yrs.	6 mos.	"Dirty wound"	Elbow	Not done	Boeck's sarcoid	Anisotropic crystals and amorphous anthracite
[5] (NMS 49–3125)	41 yrs.	W	F	33 yrs.	1 yr.	Shovel covered with cement	Forehead	Not done	Boeck's sarcoid	Anisotropic crystals
[6] (A.F.I.P. Acc. 272784)	27 yrs.	W	F	17 yrs.	2 mos.	Gravel	Elbow	Negative	Foreign body granuloma	Anisotropic crystals
[7] (A.F.I.P. Acc. 269770)	19 yrs.	Negro	M	10 yrs.	1 yr.	Brick	Forehead	Bone erosion present	Granulomatous inflammation, foreign body?	Anisotropic crystals

Case 1

A. T., a white female, 27 years old, was struck on the forehead by a piece of coal 15 years prior to admission. Since that time she had noticed a small, black area at the site of injury. This lesion was asymptomatic until 2 weeks prior to admission when it became tender but did not increase in size. No roentgenograms were taken. The lesion was excised under local anesthesia.

The gross specimen (48–4471) consisted of a formalin-fixed piece of skin and subcutaneous tissue measuring 2.5 by 1.5 by 0.5 cm. The epidermis was not ulcerated. In the dermis was an ill-defined nodule, 0.3 cm. in diameter, the cut surface of which exhibited many small, black markings. The nodule was surrounded by dense yellow-white tissue.

Case 2

A. B. was a white female, 28 years of age, who sustained a laceration of the forehead by a piece of slate roofing 15 years prior to admission. The wound was deep and was not thoroughly cleaned. About 6 weeks before admission a swelling was noted beneath the scar. Examination revealed an ill-defined, firm, non-tender, subcutaneous mass. Roentgenograms of the skull failed to reveal radio-opaque foreign material, but a small defect in the outer table of the skull beneath the mass was noted. Under local anesthesia a discrete, firm, subcutaneous nodule was excised from beneath the scar. The underlying galea aponeurotica was not seen to be involved.

The gross specimen (24564) consisted of a piece of skin and subcutaneous tissue measuring 2.5 by 2.5 by 1.5 cm. In the dermis was an ill-defined, firm, fibrous mass. A few fibers of skeletal muscle were adherent to the under surface of the specimen.

Case 3

M. D., a white female, 27 years old, sustained a 1 inch laceration to her forehead when she ran into a tree while skiing 17 years before admission. The wound healed *per primam*, leaving a barely noticeable, non-pigmented scar. One month before entry the patient noted a slightly tender swelling beneath the scar. This lesion was excised. The surgical incision healed, but shortly thereafter the swelling recurred. Three months after the first operation a firm, slightly movable mass, 2 cm. in diameter, was found on the forehead beneath the operative scar. Roentgenograms of the skull revealed no lesions in the bone. The clinical diagnosis was retention cyst. The nodule was excised together with a portion of the periosteum to which the mass was firmly attached. Healing was uneventful, and the patient has been free from symptoms for 1 year.

The specimen from the first operation (48-4421) consisted of an irregular piece of tissue, 0.3 cm. in greatest dimension.

The gross specimen from the second operation (49–521) consisted of two pieces of tissue. One was comprised of skin and subcutaneous tissue; it measured 1.6 by 1.0 by 0.8 cm. The cut surface was firm and yellow-gray. The overlying epidermis was intact. The other piece of tissue, representing an extension to the periosteum, consisted of an irregular fragment of brownish gray tissue, 0.5 cm. in greatest dimension.

Case 4

R. W. H. was a white male, 27 years of age, who received a laceration contaminated with dirt just below the right elbow which healed *per primam* after being cleaned. Twelve years later, he noted a slowly enlarging, non-tender mass at the site of injury. Six months later examination revealed a flat, well healed scar, 1 cm. in diameter, in the center of which was a blue, mottled area of pigmentation. Beneath the scar there was a soft, apparently encapsulated mass measuring approximately 4 by 3 by 3 cm. The scar and mass were removed under local anesthesia. The clinical diagnosis was chronic olecranon bursitis. The mass was described by the surgeon as "a scarred granulomatous mass of great density with small dirt particles scattered throughout."

The gross specimen (48–3639) consisted of a formalin-fixed piece of very firm, grayish brown tissue measuring 3.0 by 2.5 by 1.5 cm., which cut with a cartilaginous consistency. The sectioned surface was gray and homogeneous.

Case 5

M. A. W., a white female, 41 years old, was admitted to the U.S. Naval Hospital, Bethesda, Maryland, complaining of swelling underlying a scar over the left eye. She had been struck there 34 years prior to admission by a shovel covered with wet cement. The wound had bled freely and healed *per primam*, leaving a small scar. One year prior to admission the scar became slightly tender and more prominent. It continued to increase slowly in size until it was removed under local anesthesia.

The gross specimen (49–3125) consisted of an elliptic piece of skin measuring 1.7 by 0.8 by 0.5 cm. The epidermis was not ulcerated, but the faint outline of an old healed scar was seen. The cut surface revealed the dermis to be replaced by dense, white scar tissue.

Case 6

L. J. was a white female, 27 years old, who sustained a deep laceration on the right elbow as a result of a fall on a gravel walk at the age of 10 years. Seventeen years later a painless, non-tender swelling developed at the site of trauma; 2 months later she was admitted to the Army Station Hospital, Fairfield, California, where the lesion was excised. A roentgenogram of the skull did not show calcification or foreign body.

The gross specimen (A.F.I.P. Acc. 272784) consisted of an ellipse measuring 3 by 2 by 1 cm. In the center of the skin surface, a firm scar measuring 2 by 0.5 cm. was present, beneath which was an area of dense gray-white tissue. (This case is included through the courtesy of the Armed Forces Institute of Pathology.)

Case 7

B. W. S., a Negro male, 19 years old, was struck on the forehead by a brick at the age of 9, resulting in a transverse scar on the left frontal region. Ten years later and 1 year prior to admission, he noticed intermittent swelling of the scar, which persisted until he was admitted to Percy Jones General Hospital, Battle Creek, Michigan. Physical examination at that time revealed the scar on the forehead to be firm, non-tender, and fixed. A roentgenogram revealed bone erosion of the outer

table of the skull beneath the soft tissue mass. The scar and subcutaneous mass were removed under general anesthesia. Healing was uneventful, and the patient was returned to duty 2 weeks later.

The gross specimen (A.F.I.P. Acc. 269770) consisted of two portions, the first of which was bone chips representing an area of 1.5 to 2.0 cc. The second portion included several pieces of skin and subcutaneous tissue, the largest measuring 5.0 by 1.5 by 1.0 cm. (This case is included through the courtesy of the Armed Forces Institute of Pathology.)

Microscopic Examination of Cases 1 Through 7

Since the histologic appearance of the lesions of all 7 cases was similar, one description will suffice.

Sections stained with hematoxylin and eosin showed in the subcutaneous tissue and dermis many discrete, and occasionally confluent, non-caseating tubercles, composed of whorled epithelioid cells (Figs. 1, 2, and 3). These tubercles were separated by dense collagenous bundles and diffusely arranged epithelioid cells. In some of the tubercles were giant cells of the Langhans type (Fig. 2). Infiltration of the intervening stroma with lymphocytes and a few plasma cells was found regularly. The most striking feature was the presence of highly refractile, anisotropic, crystalline foreign bodies, often located within tubercles (Figs. 4, 5, 6, and 7). This crystalline material was irregular in shape and varied in size from being just visible under the high dry lens to approximately 100 μ in length. The larger crystals produced much scoring of the tissue where caught by the microtome knife (Figs. 4 and 6). Some of the crystals were found in the cytoplasm of giant cells of the Langhans type (Fig. 6). The overlying epidermis was not remarkable. In 2 of the cases (nos. 1 and 4), a mixture of crystalline material and black, amorphous material was present (Fig. 5). In one of these there was a history of having been struck with a piece of coal; in the other case the wound was described as dirty. In cases 2 and 7, the tubercles were not only present in the skin, but extended between skeletal muscle fibers down to the periosteum. In one case (no. 3), in some areas, there was no tubercle formation but only crystalline material embedded in dense fibrous tissue (Fig. 8). In another case (no. 7), the tubercles had undergone necrosis and were replaced by hyalinized connective tissue containing numerous crystals. About these areas was a rim of tubercles. Asteroid bodies similar to those found in Boeck's sarcoid and other granulomas were present in the cytoplasm of giant cells in 2 of the cases (Figs. 9 and 10). In none of the cases were Schaumann-Boeck bodies found. This negative finding is not of significance since only 7 cases are reported.

The sections were stained for phosphate with von Kossa's method, for calcium with alizarin, and for iron by Perls' reaction. None of the crystals in these cases showed iron, phosphate, or calcium. Sections of Boeck's sarcoid of the skin likewise were stained by these three methods and the anisotropic Schaumann-Boeck bodies showed calcium, phosphate, and iron. The Schaumann-Boeck body cannot be confused with the crystalline material in our cases because the Schaumann-Boeck body is laminated and stains blue with hematoxylin.

Special Studies

Special studies were undertaken in an attempt to characterize the crystals further.

Crystallographic Studies. Representative sections were studied under the polarizing microscope. Very little information was obtained. All of the crystals were strongly anisotropic, which serves to differentiate them from crystals of the cubic system, such as sodium chloride and other simple salts of inorganic acids, and from glass particles which are amorphous and isotropic. Due to the minute size of the crystals, it was impossible to obtain more specific identification by crystallographic technic. However, known crystals of talc (magnesium silicate) were compared, and they were likewise strongly anisotropic and similar in all respects to the crystals seen in the sections.

Spectroscopic Analysis. Tissue from cases 1, 2, and 3 was subjected to spectrographic analysis. In each case, primary and secondary lines of silicon were found at the following locations of the spectrum: primary lines at 2881.59 and 2516.12 angstroms; secondary lines at 2506.9, 2514.32, 2519.21, 2524.12, and 2528.52 angstroms.

The most dense silicon lines were found in case 2.

Semiquantitative estimation determined that silicon was a major inorganic constituent of the tissue examined. However, Brown [4] reported that silicon may be present in normal skin in amounts as high as 0.045 to 0.055 mg. per gm. of skin. Therefore, these determinations are not significant.

Micro-Incineration. Paraffin sections of the tissues from cases 1 through 5 were micro-incinerated and treated with 5 per cent HCl in an effort to identify the crystals as silica. However, due to the relatively small quantity of crystalline material present in the tissue, and the possible loss of the crystals during cutting and micro-incineration, the results were inconclusive.

Solubility Studies with Acids. Frozen sections of the tissue from case 5, which contained abundant crystalline material, were treated with 5 per cent and 38 per cent hydrochloric acid, and 48 per cent

hydrofluoric acid. Examination of these sections at varying intervals revealed the crystals to persist in the tissue sections following the addition of the hydrochloric acid; but they disappeared following the addition of hydrofluoric acid. This is further evidence that the crystalline material is siliceous in nature.

Discussion

We believe these 7 cases constitute a clinical and pathologic entity, which in the past has not been clearly defined. The lesions all occurred in young patients, who, many years prior to the onset of symptoms, sustained a laceration contaminated by foreign material. The foreign agents included coal, slate, cement, gravel, brick, and earth, all of which contain silica. The lacerations healed without difficulty, but the patient developed symptoms many years later. This latent interval varied from 10 to 33 years and averaged 17 years. Despite this long latent interval there was a rather acute onset of symptoms, varying from 2 weeks to 1 year, with an average of 5 months. The symptoms consisted of the development of a small, usually slightly tender, firm mass beneath an old scar. The only sites in our cases were over the forehead and elbow. Roentgenograms did not reveal the foreign material but showed erosion of the outer table of the skull in 2 of the cases. The surgeon was often in doubt as to diagnosis, and in a few cases found the operative procedure more difficult than contemplated since the granulomatous reactions extended through muscles to involve periosteum. The histologic appearance was strikingly similar in all cases. After complete excision, the patients have remained well. In one case, in which all of the crystalline material was not removed, there was prompt recurrence; after re-excision the patient has remained well.

These granulomatous responses to foreign crystalline material may be readily mistaken for Boeck's sarcoid of the skin, as attested by this erroneous diagnosis in 4 of the 7 cases. Although both Boeck's sarcoid and these granulomas contain non-caseating tubercles, giant cells of the Langhans type, anisotropic, intracellular, crystalline material, and occasional asteroid bodies, the differential diagnosis may be made with relative ease by study of the crystalline material. The crystalline material in these granulomas is often coarse, colorless, non-laminated, spiculated, and gives a negative reaction to stains for iron, calcium, and phosphate. In contrast, the anisotropic Schaumann-Boeck body is small, round, laminated, stains blue with hematoxylin and eosin and positively for iron, calcium, and phosphate. Schaumann-Boeck bodies are absent in many cases of Boeck's sarcoid. It is believed that the mistaken diagnoses have been the result of lack of awareness of this entity and the

belief that the foreign crystalline material represented artifact, or Schaumann-Boeck bodies.

Gardner[5] has demonstrated that silica is tubercle-producing both in animals and man.

Spectrographic, crystallographic, and micro-incineration technics furnished no specific or conclusive data, but gave results compatible with the *a priori* hypothesis that the material was either silica or a silica-containing substance. That these lesions represented a reaction to silica is supported by the history of trauma with diverse silica-containing materials and the similarity of the lesions to talc granulomas (first described by Antopol,[6] and later by German[7]), silicosis of the lungs,[8] and beryllium granulomas.[2] Some of the cases contained black, amorphous material in addition to crystalline material. When the amorphous substance was unassociated with crystalline material there was no epithelioid cell response. Additional support for the theory that these 7 cases represent a type of traumatic silicosis is that a long latent interval between introduction of the foreign material and the development of symptoms also obtains in silicosis of the lungs.

Evans and Zeit[8] have proposed the "piezo-electric theory" for explaining the production of silicosis. This is based on the principle that if a mechanical force is applied to two opposite faces of a silica crystal, an electric charge appears on the other two faces; conversely, an electric charge applied to two opposite faces will cause mechanical distortion of the crystal. They believe that the fibrosis provoked by silica particles represents a tissue response to a recurrent mechanical or electrical insult caused by the piezo-electric effect. It is of interest that the lesions in our cases occurred either on the forehead, or elbow, two sites subject to frequent motion, and thus subject to the piezo-electric effect.

Conclusions

In the tissues removed from 7 patients with post-traumatic subcutaneous granulomas a crystalline material was present. The foreign material was probably silica.

These cases present a clinical and pathologic entity characterized by trauma with silica-containing material, with laceration of the skin healing *per primam*, a latent interval of many years, and a rather acute onset of an often tender nodule beneath the scar, which on pathologic examination reveals non-caseating tubercles, giant cells of the Langhans type, and anisotropic crystalline material.

These granulomatous lesions have been mistaken for Boeck's sarcoid of the skin, but may be differentiated by the appearance of the crystalline material, and by special stains.

We wish to thank Dr. Benjamin J. Highman, National Institutes of Health, Bethesda, Md., for micro-incineration studies; Dr. Leonard Fairhall, National Institutes of Health, Bethesda, Md., for spectrographic analyses; and Dr. Elson B. Helwig, Armed Forces Institute of Pathology, Washington, D.C., and Lt. E. E. Ozburn, M.S.C., U.S.N., Naval Medical School, Bethesda, Md., for helpful suggestions.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 52

FIG. 1. Case 5 (49–3125). Confluent subcutaneous tubercles composed of epithelioid cells. × 30.

FIG. 2. Case 5 (49–3125). Discrete tubercles containing giant cells of the Langhans type, surrounded by dense connective tissue. × 100.

FIG. 3. Case 4 (48–3639). Discrete tubercles in subcutaneous tissue. There is lymphocytic infiltration in intervening connective tissue. Resemblance to Boeck's sarcoid may be noted. × 100.

FIG. 4. Case 3 (49–521). Subcutaneous tubercles composed of epithelioid cells and a large crystalline fragment. Of note is the scored tissue where the crystal was caught by the microtome knife. × 200.

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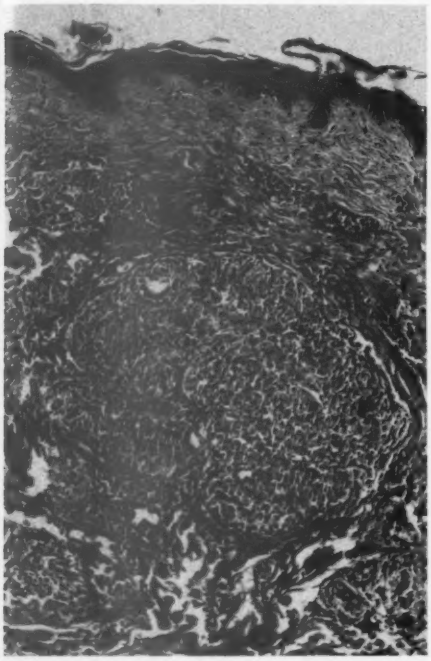

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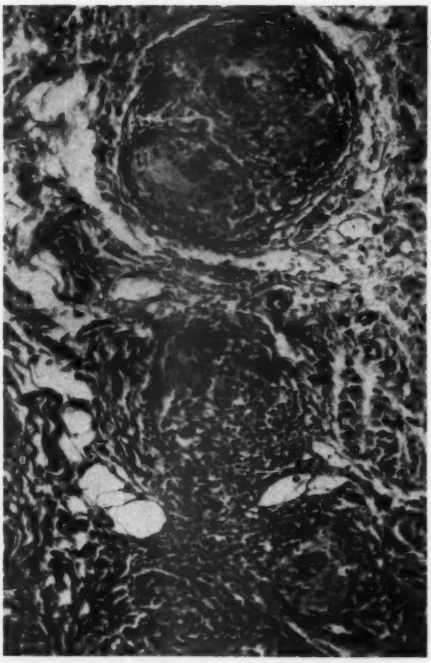

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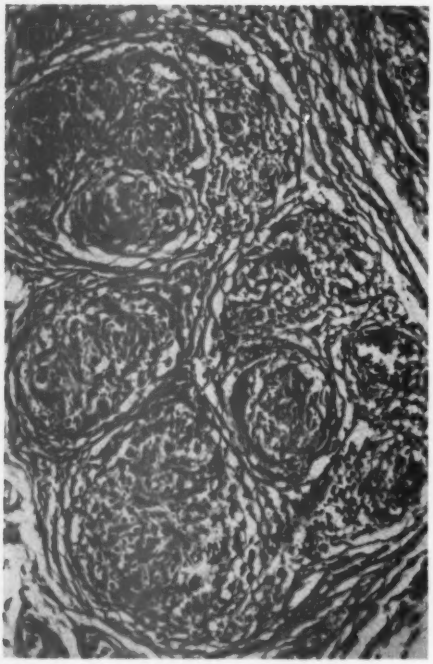

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Ayres, Ober, and Hamilton

Post-Traumatic Subcutaneous Granulomas

Plate 53

Fig. 5. Case 1 (48–4471). Amorphous black material and crystalline material within a tubercle. × 200.

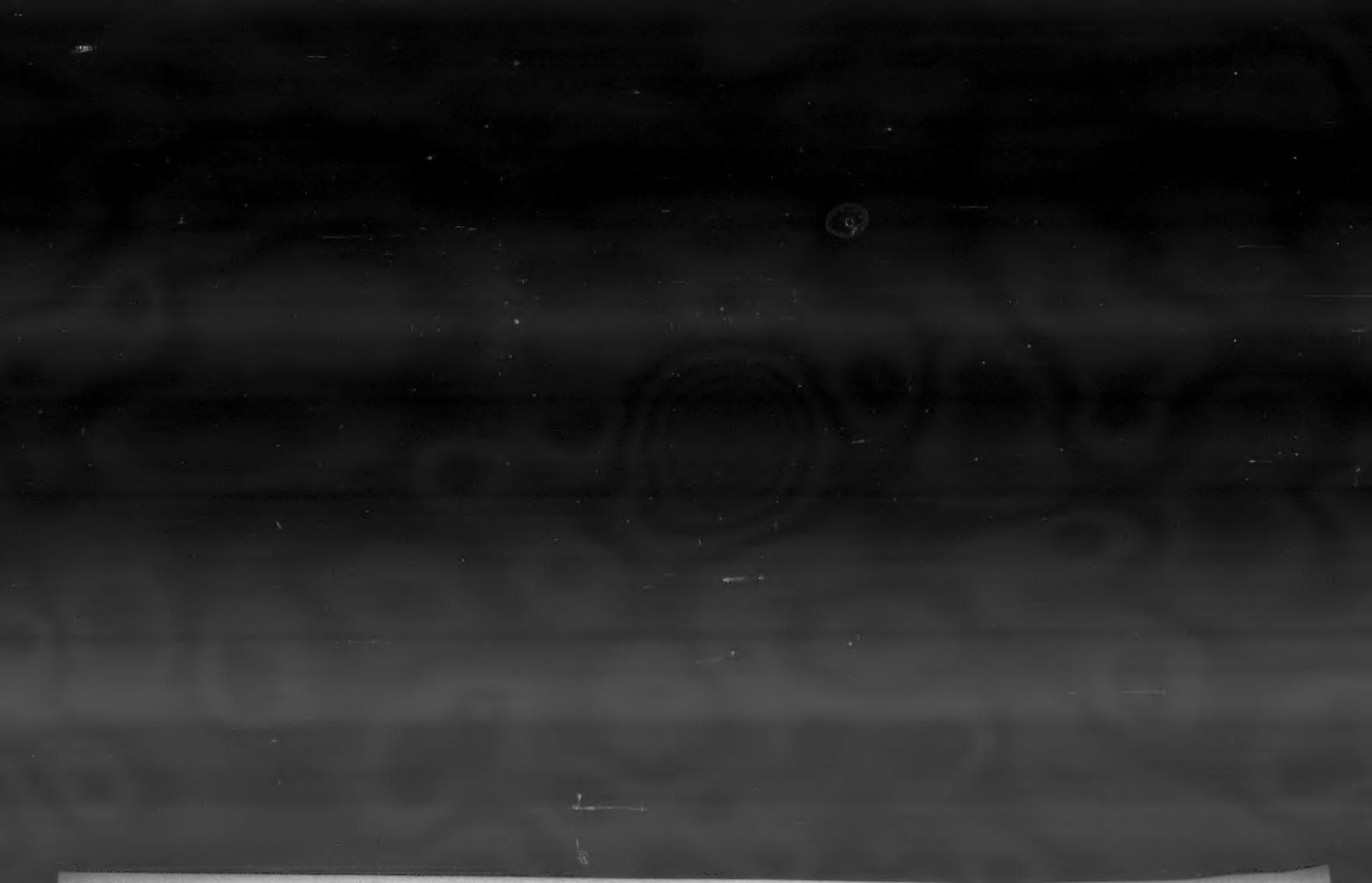
Fig. 6. Case 2 (24564). Crystalline material in the cytoplasm of a giant cell of the Langhans type. × 200.

Fig. 7. Case 3 (49–521). Photograph made with the polarizing microscope to exhibit many large and small anisotropic crystals. × 150.

Fig. 8. Case 3 (49–521). Crystalline material embedded in dense connective tissue. Tubercles are absent. × 200.

Fig. 9. Case 1 (48–4471). Giant cells of the Langhans type containing an asteroid body in the cytoplasm. There is also tubercle formation and an infiltration with lymphocytes. × 200.

Fig. 10. Detail of Figure 7. × 400.



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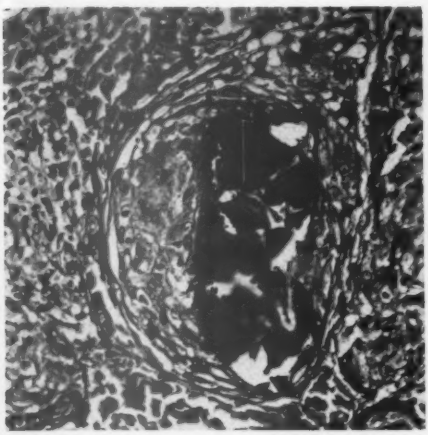

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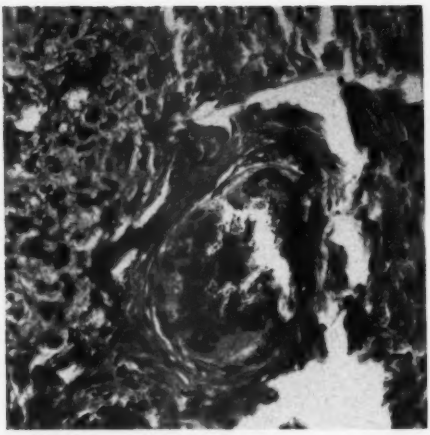

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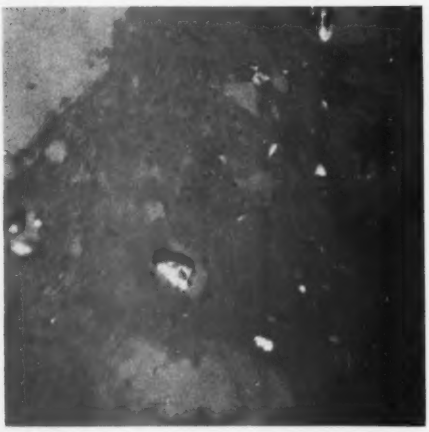

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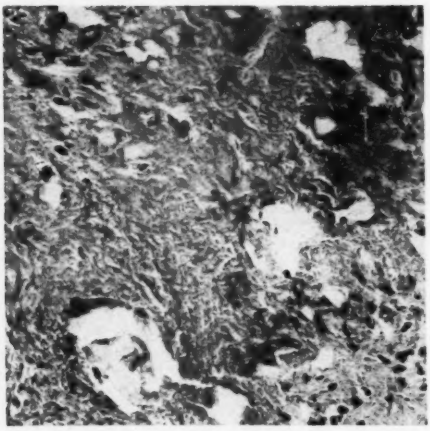

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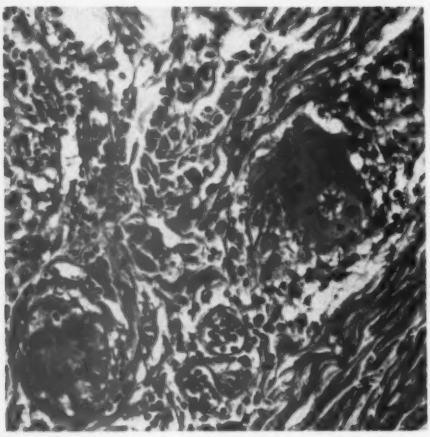

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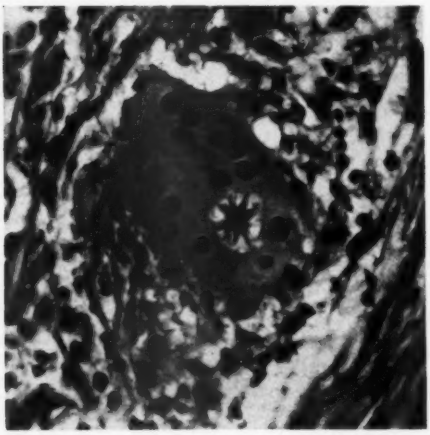

10

Ayres, Ober, and Hamilton Post-Traumatic Subcutaneous Granulomas

GIANT CELL FOREIGN BODY REACTION TO NON-CHOLESTEROL LIPID PLATE CRYSTALS

A Case Report with Chemical Studies*

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There have been numerous reports of foreign body reaction in which plate crystal slits have been a prominent feature of the granulomas and the reaction was assumed to be due to cholesterol crystallized in the tissue. This is the report of a case in which giant cell granulomatous lesions with plate crystal slits were found in the lungs and the crystals were shown to be lipid but not cholesterol or an ester of cholesterol.

Report of Case

The patient was a white female, 68 years old, who, 1 year before death, developed asthma with "wheezing," slight dyspnea, and a cough productive of small amounts of gray sputum. There was a weight loss of 10 lbs. When examined at the New York Hospital, her temperature was 38° C.; respirations, 20 per minute; blood pressure, 163/100 mm. of Hg. She appeared chronically ill. The trachea was deviated markedly to the right. Physical examination and roentgenograms of the chest suggested a differential diagnosis between tuberculosis and carcinoma of the upper lobe of the right lung. Shortly after a bronchoscopic examination under local cocaine anesthesia, the patient died suddenly in respiratory distress.

At necropsy, the lungs together weighed 1350 gm. There were dense fibrous adhesions from the upper and middle lobes of the right lung to the chest wall and between the lobes, completely obliterating the upper portion of the pleural cavity and pulling the trachea to the right. The right upper and middle lobes of the lungs were small, purple, rubbery hard, and, on section, gray-green. No alveoli could be identified. The other lobes of the lungs appeared normal. There were a few small areas of pleural thickening at the apex of the left lung. The hilar and tracheobronchial lymph nodes were not enlarged.

On microscopic examination the lesion in the upper and middle lobes of the right lung resembled a foreign body reaction. The alveolar structure was almost completely obliterated by moderately dense fibrous tissue containing foci of lymphocytes, monocytes, and foamy macrophages (lipophages), as well as numerous large giant cells of foreign body type without any surrounding epithelioid cells. A number of the giant cells contained stellate asteroid bodies (Fig. 7), a few cells containing as many as three or four. Many of the giant cells were arranged

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in groups around several elongated, spindle-shaped slits. Slits were located also within some of the giant cells (Figs. 1, 2, and 3). A frozen section stained with sudan IV revealed plate crystals in place of the slits seen in paraffin sections. Some of the macrophages contained numerous tiny globules of fat, stainable with sudan IV. Acid-fast, Giemsa, and Gram stains revealed no organisms. Sections of the remaining lobes of the lungs revealed moderate hemorrhage and edema. There was a slight bronchopneumonia of the lower lobe of the right lung (beta hemolytic streptococcus cultured post mortem). A few small foreign body granulomas, as described above, were seen also in the lower lobe of the right lung and in the thickened visceral pleura of the upper lobe of the left lung. A section through a hilar lymph node in the upper lobe of the right lung revealed moderate numbers of macrophages and red blood cells in the sinuses as well as considerable black and golden-brown pigment. No granulomas were seen.

Other significant lesions included obliterative fibrous pericarditis (no granulomas demonstrable), and moderate congestion of the liver and spleen. Death was attributed to hypersensitivity to cocaine.

Chemical Studies

Sufficient material was available for tests on the nature of the plate crystals. When unstained frozen sections of the lung were made without running the sections through the usual fat solvents, a crystalline material was present in place of the previously seen slits (Fig. 4). Under a dissecting microscope at 72 ×, it was possible to dissect out the clumps of crystals from the formalin-fixed tissue blocks and place them on a slide (Fig. 5).

Text-figure 1. Plate crystal showing measured angles.

The angles of one of the perfectly formed plate crystals were measured. Text-figure 1 is a diagram of the crystals. The crystals were anisotropic, melted at 77° to 78°C. (microscopic technic), and gave a strongly positive Liebermann-Burchard reaction. It was possible to test the solubility of the crystals by applying a drop of solvent to them on a slide. Using this technic, they were found to be soluble at room temperature in ether, chloroform, carbon tetrachloride, benzene, hexane, xylol, toluol, and hot ethanol; and insoluble in water, cold ethanol, acetone, methanol,

N/10 hydrochloric acid, N/10 sodium hydroxide, sodium bicarbonate solution, dilute and glacial acetic acid, and ethylacetate. In relatively much larger quantities of acetone the crystals were soluble.

On the basis of solubility, extraction of the crystals from the fibrosed lung tissue that had been fixed in formalin was attempted. The lung tissue was minced and dried on a paper towel. Twenty grams of tissue were added to 20 cc. of cold ethanol and the mixture placed in the refrigerator for 4 days, at which time the ethanol was poured off and discarded. Twenty cc. of ether were added to the lung tissue and kept at room temperature for 4 days, the evaporating ether being replaced. The ether solution was washed with water in a separatory funnel, filtered, and then placed at room temperature to evaporate. When half of the ether had evaporated, a drop of solution was placed on a slide, the ether quickly evaporating and leaving behind small, round droplets of an oily substance. The plate crystals expected were not seen so the slide was placed aside. Two hours later the slide was again examined under the microscope. In each droplet of oil there was a small, almost perfectly shaped, crystalline asteroid body, some of the larger areas of oil containing several bodies (Fig. 6). In some there was a round, central body from which radiated thin, needle-like spicules, some of the spicules being gracefully curved as in the asteroid bodies seen in the tissue. The melting point of the crystals was found to be 76° to 77° C. (microscopic technic). The crystals were doubly refractile, soluble in ether and xylol, insoluble in cold ethanol, and relatively insoluble in acetone.

Repeated attempts to get similar crystals using similar technics sometimes were successful and sometimes not. Most often needle crystals appeared but did not have the shape of a typical asteroid, many of the crystals branching out unevenly in all directions, forming a tree-like structure. On several occasions, however, typical asteroids were formed. Since these crystals looked so strikingly like the asteroid bodies seen in the giant cells, the asteroid bodies in unstained frozen tissue sections were examined. Some of the same giant cells that contained the plate crystals contained as many as three or four asteroid bodies. The asteroid bodies, however, did not seem to have a crystalline structure, but rather had the appearance of condensed cytoplasm in stained and unstained preparations (Fig. 7). These bodies were not doubly refractile, in contrast to the doubly refractile plate crystals.

Further attempts were made to obtain plate crystals from the ether extract. When the ether had completely evaporated, a light yellow, amorphous material remained. Enough ether was then added to dissolve all of the material completely. Five times that amount of acetone was added and the solution kept at room temperature for evaporation. The

total volume was 20 cc. After 1 week all but 4 cc. of liquid had evaporated, leaving behind small clumps of white crystals which, under the microscope, had the same appearance as the plate crystals dissected from the lung. The crystals were washed with acetone and recrystallized several times in this manner. The crystals had a melting point of 77°C. (microscopic technic) and gave a strongly positive Liebermann-Burchard reaction. The ammonium molybdate-amidol spot test for phosphorus was negative after prolonged hydrolysis of the crystals with hot, concentrated sulfuric acid.

The material was dissolved in carbon disulfide, and the infra-red absorption spectrum was obtained.* The absorption spectrum in the region from 1180 to 750 cm.$^{-1}$ exhibited absorption bands which were not characteristic of those given by cholesterol or cholesterol acetate. The material was recovered and submitted to hydrolysis with a mixture of alcohol, acetone, and normal potassium hydroxide at 50° C. This method would lead to the recovery of free cholesterol, if present as one of the cholesterol esters obtained from blood or tissue. Infra-red analysis of this material gave a spectrum which was different from that given by the original material and also was different from that given by cholesterol. This strongly suggests that the material submitted was neither cholesterol nor a known cholesterol ester.

A drop of the supernatant fluid was placed on a slide. The liquid quickly evaporated, leaving behind small oil droplets. After 1 hour, numerous very small plate crystals having the appearance of the crystals dissected out of the lungs had formed (Fig. 8). A few asteroid crystals were seen also. In one or two instances a single crystal seemed to be forming into both a plate and an asteroid.

Discussion

From the chemical studies it has been impossible to identify the plate crystals. The solubilities seem to indicate that the substance is a lipid, and the strongly positive Liebermann-Burchard reaction, if not due to impurities, would indicate a steroid. The infra-red absorption spectrum before and after hydrolysis was not that of cholesterol or an ester of cholesterol. The best possibility would seem to be that the substance is a steroid but not cholesterol or an ester of cholesterol.

That there is a possible relationship between the plate crystals and the produced asteroid crystals can be seen from the similarities in their properties. They have similar melting points and solubilities. In addition, the two crystal forms were seen to originate in a few instances

* I am indebted to Dr. K. Dobriner and Mrs. P. Humphries for data on the infra-red absorption spectra and their interpretation. The value in the following sentence expresses frequency and not wave length.

from the same nucleus. It is probable that the two types represent two crystal forms of the same substance. Whether these asteroid crystals are related to the asteroid bodies seen in the tissue can only be surmised. A number of substances can crystallize in needle form, producing structures similar to asteroids.[1] However, in this instance it seems highly probable that the same substance that caused the foreign body reaction could also be responsible for the production of the co-existing asteroid bodies, especially since it has been demonstrated that it can crystallize out in such a characteristic manner. It should be remembered, however, that such doubly refractile asteroid crystals are not seen in the asteroid bodies of giant cells when they are viewed in unstained frozen sections. Rather, the asteroid bodies have the appearance of condensed cytoplasm.

Stahel-Stehli [2] reported a case of cholesterol foreign body granuloma of the lung in diabetes mellitus. The lesions pictured were identical with those described here. On the basis of extracting excess cholesterol from the tissues, the author concluded that the crystals in the giant cells were cholesterol. No attempts were made to identify the foreign crystals directly.

Hirsch,[3] in 1935, gave an excellent review of the literature. He was the first to work on the chemistry of the asteroid bodies. He concluded that asteroid bodies are crystalline forms of fats which are solid at body temperature, such as palmitin and stearin, separated from an oil system containing cholesterol or a similar substance. The conclusions were based on the known cholesterol-fat relationship in tissues; on chemical analysis of the spleen containing asteroids and finding excess stearin and palmitin; and on the production in lungs of rabbits of a foreign body reaction to rosette-shaped crystals following intravenous injections of cholesterol-palmitin mixtures. Furthermore, he concluded that the formation of the asteroid crystals is according to the usual rules of crystallization. Chemical changes occur in the tissues so that the crystals are no longer soluble in fat solvents and stain with elastin stains. Friedman,[4] in 1944, concluded that asteroid bodies give no specific staining reaction and have the same staining properties as cytoplasm of endothelial or giant cells. They are not disease-specific since they have been seen in cases of talcum powder granuloma,[5] leprosy,[6] and in the tophi of gout.[6]

Summary

In the material from a patient with pulmonary foreign body giant cell granulomas, plate crystal slits and asteroid bodies were found. The plate crystals have been shown to be a lipid, possibly a steroid, but

even though they gave a positive Liebermann-Burchard reaction, were not cholesterol or an ester of cholesterol. A possible relation between the plate crystals and the asteroid bodies is suggested by observations made in the course of this study.

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DESCRIPTION OF PLATES

PLATE 54

FIG. 1. Lung, demonstrating foreign body giant cell granuloma with plate crystal slits. Hematoxylin and eosin stain. × 145. (Armed Forces Institute of Pathology Neg. 104567.)

FIG. 2. Lung, demonstrating foreign body giant cell granuloma with plate crystal slit and asteroid body in giant cell. Hematoxylin and eosin stain. × 285. (A.F.I.P. Neg. 103535.)

FIG. 3. Lung, demonstrating foreign body giant cell granuloma with plate crystal slits. Hematoxylin and eosin stain. × 285. (A.F.I.P. Neg. 103536.)

FIG. 4. Lung. Unstained frozen section fixed in formalin, demonstrating crystalline material (c), and asteroid bodies (a) in giant cells. × 450. (A.F.I.P. Neg. 103356.)

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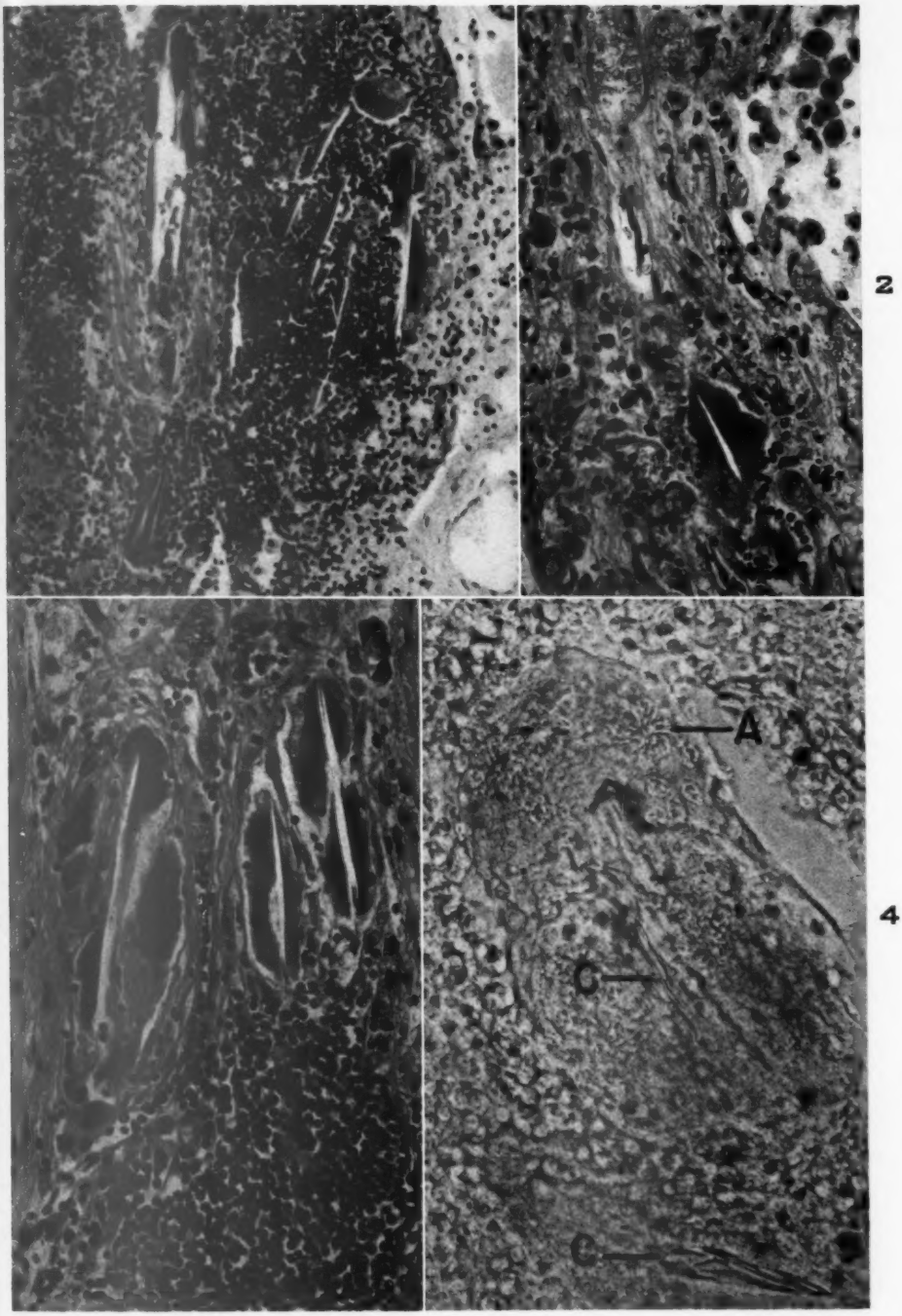


Engle

Giant Cell Reaction to Non-Cholesterol Crystals

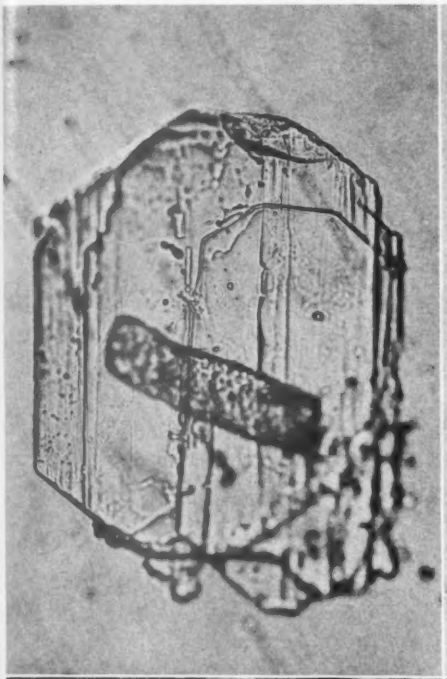
PLATE 55

FIG. 5. Plate crystals dissected from the granulomas in the lung. Unstained. × 315. (A.F.I.P. Neg. 103362.)

FIG. 6. Asteroid crystals obtained by extraction from the tissues of the lung. Unstained. × 450. (A.F.I.P. Neg. 103360.)

FIG. 7. Asteroid body in a giant cell. Hematoxylin and eosin stain. × 900. (A.F.I.P. Neg. 103347.)

FIG. 8. Plate crystals obtained by extraction from the tissues of the lung are similar in shape to the crystals dissected from the lung and pictured in Figure 5. Unstained. × 450. (A.F.I.P. Neg. 103359.)



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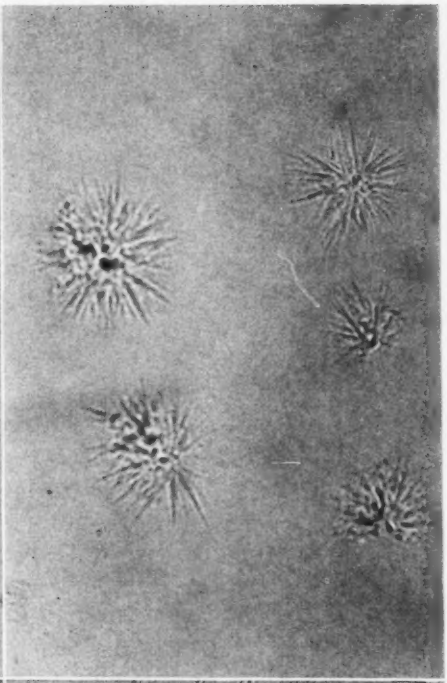

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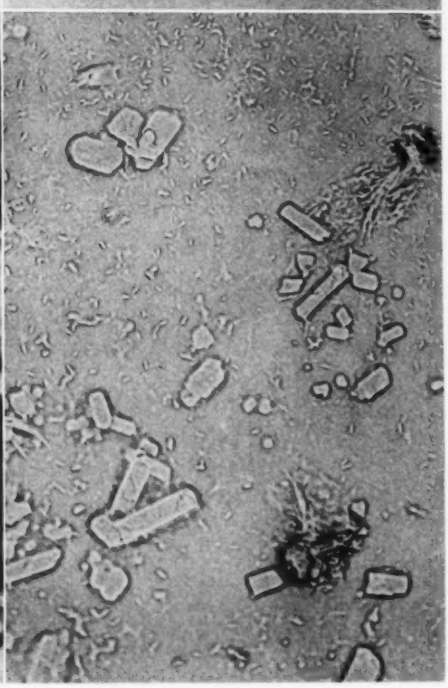

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Engle

Giant Cell Reaction to Non-Cholesterol Crystals

GLYCOGEN INFILTRATION (SO-CALLED HYDROPIC DEGENERATION) IN THE PANCREAS IN HUMAN AND EXPERIMENTAL DIABETES MELLITUS *

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In 1901 Weichselbaum and Stangl [1] described unique changes in the islets of Langerhans of 18 pancreases from comatose diabetic human subjects. Having found the affected islet cells uncolored by methods designed to demonstrate hyaline and mucoid forms of degeneration, they [2] later interpreted the lesion as a manifestation of cytoplasmic vacuolation and liquefaction, and applied the term hydropic degeneration to indicate its presumably aqueous character. Allen [3] accomplished the first experimental production of a similar lesion in the islets and ductules of the pancreatic remnants of dogs made diabetic by resection of nine-tenths of the pancreas. Homans [4,5] demonstrated that in the islets of such experimental material, degranulation and vacuolation developed only in the beta cells. Both experimenters failed to find fat, mucin, or glycogen in the affected cells; each concluded, as Weichselbaum did, that the vacuoles represented aqueous fluid. Richardson,[6] Ham and Haist,[7] Lukens and Dohan,[8,9] and Lukens, Dohan, and Wolcott [10] described the pancreases of dogs and cats treated with anterior pituitary extract; these authors apparently accepted the idea of aqueous vacuolation as the basis of "hydropic degeneration" in their material.

Kennedy and Lukens,[11] Duff, McMillan, and Wilson,[12] and Ogilvie [13] reported "hydropic degeneration" in the pancreas of alloxan diabetes of rabbits, and Goldner and Gomori [14] observed it in alloxan diabetes of dogs. Duff, McMillan, and Wilson did not find fat, mucin, or glycogen in the vacuolated islet and ductule cells, while Ogilvie attributed the lesion to artifactual loss of excessive intracytoplasmic accumulations of serous fluid. Dohan and Lukens [15] produced permanent diabetes and pancreatic islet "hydropic degeneration" in cats by repeated intraperitoneal injections of glucose. Kobernick and More [16] recently observed a severe diabetic state in rabbits given repeated injections of cortisone. In the pancreases of some of these animals extreme "hydropic" appearances were evident in the beta islet cells, ductular epithelium, and centro-acinar cells.

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Pathology textbooks generally attribute the cytoplasmic vacuolation of "hydropic degeneration" to an exaggerated process of aqueous imbibition qualitatively similar to the supposed mechanism of cloudy swelling. Except for the presence of glycogen in fetal pancreatic duct epithelium, as reported by Ohohashi,[17] the normal human pancreas has not been reported to contain histologically demonstrable glycogen. Warren [18] has observed glycogen deposits in the cuboidal and columnar epithelium of pancreatic ducts in human diabetes mellitus, but he has never found it in any other pancreatic cells. He apparently regarded these deposits as simply another occasional site of abnormal glycogen infiltration comparable to the others often encountered in various tissues of diabetic subjects.

The vacuolated islet cells of partially depancreatized diabetic dogs, of dogs and cats rendered diabetic by injections of anterior pituitary extract, and, according to Weichselbaum,[19] of diabetic human beings, gradually disintegrate and disappear. However, the affected ductular cells of diabetic dogs and both the islet and ductular cells of diabetic rabbits persist in the vacuolated state for many weeks or months. Such cells may be restored to normal appearance by appropriate manipulation of the carbohydrate content of the diet,[9,20] by poisoning with phloridzin,[10] or by the provision of exogenous insulin.[8,9,12,21,22] This capacity for prolonged persistence in a remarkably abnormal morphologic state which is nevertheless reversible would seem to be incompatible with the generally accepted presumption that the cytoplasmic vacuoles contain nothing but excessive quantities of water. Furthermore, the presence of mitochondria, as noted by Homans,[4,5] and the Golgi apparatus and macular area, as demonstrated by Bencosme,[23] in "hydropic" islet cells, is irreconcilable with the aqueous imbibition hypothesis. The structural features of various cells known to contain large quantities of cytoplasmic glycogen (*e.g.*, vaginal epithelial cells, parathyroid "wasserhelle" cells, heart muscle fibers adjacent to areas of infarction, renal tubular epithelial cells in Armanni's lesion, and liver cells in von Gierke's disease) are similar to those of pancreatic islet, ductular epithelial and centro-acinar cells affected by so-called hydropic degeneration. It is the purpose of this paper to report that these "hydropic" pancreatic cells actually do contain glycogen in amounts demonstrable by appropriate histologic methods.

Materials and Methods

Large numbers of blocks of pancreatic tissue were obtained from 20 domestic albino rabbits made permanently diabetic by single intra-

venous injections of alloxan, as described by Duff, McMillan, and Wilson.[12] Some of these animals had received repeated daily injections of protamine zinc insulin.[22] The pancreases of 2 dogs which had received repeated daily injections of crude alkaline extract of fresh frozen ox anterior pituitary glands,* prepared and used according to the methods of Young,[24] were studied. Pancreatic remnants were available also from 2 dogs subjected to extensive resection of the pancreas in the manner of Allen's [3] and Homans' [5] experiments. Drs. Kobernick and More [16] kindly allowed me to study sections of pancreas fixed in Zenker-formol (20 per cent formalin in Zenker's base) from a cortisone-treated rabbit. Some of these tissues were taken for biopsy, but the majority were secured at autopsy performed immediately after killing each animal. Small thin slices of pancreas were immersed in freshly prepared Helly's fluid (5 per cent formalin in Zenker's base), allowed to fix for 8 to 24 hours, washed in tap water for 8 to 24 hours, dehydrated in alcohol, cleared in toluol, and embedded in paraffin. Multiple sections were cut at 3 to 5 μ, and mounted on glass slides with gelatin.

The human material was obtained from autopsies performed between the years 1926 and 1950 on 26 cases of diabetes mellitus in which death occurred in coma. These tissues had been fixed in 10 per cent formalin and embedded in paraffin. New sections were cut at 5 μ. One case provided material which had been fixed in acetic acid-Bouin's fluid and embedded in paraffin. For this material, a thin film of celloidin was applied to the sections after they had been mounted on glass slides, according to the suggestion of Lillie.[25]

Blocks of rabbit kidney from alloxan diabetic animals, fixed in Helly's fluid and embedded in paraffin, provided "known-positive" control material for glycogen staining reactions and enzymatic digestion tests. Digestion was carried out for ½ to 1 hour at 37°C. in buffered 1 per cent malt diastase U.S.P.[25] All other technical manipulations of control and test sections were identical and simultaneous.

The animal tissues were stained by the following methods: hematin, phloxine, saffron; a modification of Gomori's chromic alum hematoxylin in which the ponceau mixture of Masson's trichrome was substituted for phloxine; Best's carmine; Mayer's mucicarmine; aqueous periodic acid-Schiff's reagent routine of McManus [26]; the alcoholic version of the same test described by Hotchkiss [27]; the Bauer chromic acid-Schiff's reagent technic as described by Lillie [25]; and Mancini's [28] iodine reactions. The human tissues were submitted to most of the same battery

* The pituitary glands were made available to me by the courtesy of Dr. John R. Mote, Armour & Company, Chicago, Illinois.

of stains except that efforts to demonstrate islet cell cytoplasmic granules were not made routinely.

The glycogen content of the pancreas of an alloxan diabetic rabbit in which severe "hydropic change" was present was determined chemically * by the Good, Kramer, and Somogyi method as utilized by Venning, Kazmin, and Bell.[29]

Observations

In sections stained by the hematin, phloxine, and saffron method and by the Gomori and Masson technics (Figs. 5, 13, 16, and 19), pancreatic cells affected by so-called hydropic degeneration appeared slightly to extremely swollen, being apparently distended by accumulation in their cytoplasm of a substance not visible in such preparations. The cytoplasmic membranes were sharply defined and the nuclei had well preserved, apparently normal, structural patterns. In most instances the nuclei were situated in the centers of the cells or were only slightly displaced toward the periphery; they never appeared compressed in the semilunar fashion of cells containing fatty cytoplasmic masses. Often the demonstrable cytoplasm was represented by occasional delicate wisps of lacy, cobweb-like material extending in radial fashion from the nucleus to the cytoplasmic membrane. However, there was present sometimes a more or less vesicular cytoplasm devoid of demonstrable specific beta granules but containing mitochondria, Golgi apparatus, and macular area.[23] In some cells one or several small, round or irregular, homogeneous bodies (the Körner of Weichselbaum) were evident.

Mucin was encountered frequently in epithelial cells lining the large and intermediate pancreatic ducts. The Gomori method demonstrated this material in the same situations and quantities as did the Mayer mucicarmine technic, but depicted it as fine, closely packed, blue granules. Both the McManus and the Hotchkiss versions of the periodic acid-Schiff's reagent technics colored mucin less brilliantly red than they did glycogen. Mucin-filled columnar cells of ducts often were found together with cells having vacuolated cytoplasm. In no instance was mucin detected in cells of the islets (Fig. 12). Exposure to diastase did not remove mucin.

When the pancreases of alloxan-treated rabbits were stained by the several technics capable of demonstrating glycogen, this substance was always found in the cells affected by so-called hydropic degenera-

* I am indebted to Dr. E. H. Venning of the University Clinic, Royal Victoria Hospital, for this analysis.

tion (Fig. 10). It was present not in traces but in abundance, and its location coincided accurately with the cytoplasmic vacuolar change in beta cells of the islets, ductular epithelium, and centro-acinar cells. Ordinarily there was an obvious ipsilateral shift of the material referable to the artifact attributed to the diffusion current of the fixative. Although the material was often represented as granular deposits of variable dimensions, in many cells the whole cytoplasmic area was occupied by a single, homogeneous mass which partially obscured the nucleus. In some instances there appeared to be partial loss of glycogen so that the remnant occupied only a fraction of the cytoplasmic area of the affected cells. This appearance was not more frequent than the similar and more familiar aspect of glycogen infiltration of vaginal epithelium, myocardial fibers, and renal tubular epithelium (Figs. 7, 8, and 9). Pancreatic tissue from dogs treated with anterior pituitary extract and from partially pancreatectomized dogs showed glycogen infiltration of beta islet cells, ductular epithelium, and centro-acinar cells similar in every qualitative respect to the appearance of the lesion in alloxan diabetic rabbit tissue (Fig. 14). However, the lesions observed in these experiments were of mild degree.

The presence of glycogen in the vacuolated pancreatic cells was displayed in flamboyant manner in the preparations from the cortisone diabetic rabbit (Fig. 17). The effect was exaggerated comparatively by the presence of more numerous beta cells than appeared in alloxan diabetic rabbits, so that the islet lesion, as well as the ductular one, was obvious at a glance.

If allowance be made for the poor technical quality of the available human material, the appearance of vacuolation of islet cells in it closely resembled the experimental lesions (Fig. 19). In this material, however, the glycogen appeared much more granular and was less strictly confined to cytoplasmic vacuoles (Fig. 20). Some of it appeared to have been deposited upon the islet rather than within swollen beta cells. Ductular epithelium was relatively uninvolved, even in the presence of marked infiltration of islets. Among the 26 human cases studied, "hydropic degeneration" was apparent according to ordinary stains in 11 (42.3 per cent). Glycogen was demonstrable in abundance in one case (acetic-Bouin fixation), in moderate amounts in 2, and in small amounts in 6. No positive staining reaction could be elicited in the remaining 2 instances. In no case was glycogen found in the absence of definite cytoplasmic vacuolation.

Exposure of control sections to diastase always removed the demonstrable substance from the various cells (Figs. 11, 15, 18, and 21) in

which it could regularly be demonstrated if the tissues were not exposed to diastase (Figs 10, 14, 17, and 20). Mucin in columnar duct epithelium remained demonstrable despite diastase treatment.

The pancreas of the alloxan diabetic rabbit contained 0.0025 gm. of glycogen per gm. of tissue (= 0.25 per cent).

Discussion

The lesion as described is qualitatively similar in every morphologic respect in all of the tissues studied without regard to species or mode of production of diabetes. Modifications of it are known to occur in cats, dogs, and probably in human beings, referable to gradual pyknotic and lytic changes in the affected cells terminating in numerical atrophy of beta cells. In the alloxan treated rabbit very few islets contain many glycogen-filled cells because of the preceding necrosis of the beta cells. The cortisone treated rabbit provides the most abundant and flagrant lesions; in this instance, however, the details of the development, progress, and termination of the lesion are not yet available. The amount of glycogen determined by chemical analysis is small, but it is similar to the amounts found in human kidneys in glycogen nephrosis.[18]

Artifactual removal of the abnormal content of glycogen in the cytoplasm of swollen pancreatic islet, ductular epithelial, and centro-acinar cells showing "hydropic degeneration" is probably more responsible for the characteristic vacuolation apparent in ordinary histologic preparations than is the concomitant presence of excessive intracytoplasmic water. The granular appearance of glycogen in chemically fixed preparations and the ipsilateral disposition of more homogeneous crescentic masses of intracytoplasmic glycogen represent fairly constant artifacts.[28] Fixation by freezing, dehydration *in vacuo*, and subsequent direct embedding in paraffin permit demonstration of glycogen evenly distributed throughout the cytoplasm of cells of various types which normally contain or store it.[28] Microscopic demonstrations of glycogen prepared by other technics inevitably misrepresent the amounts and locations of the material to some extent. Because in the normal process of glycogen storage in the liver each gram of glycogen stored is accompanied by the simultaneous entrance of 1.16 to 2.33 cc. of water into the liver cells,[30] it is reasonable to suppose that a similar imbibition may occur when abnormal deposits of glycogen accumulate in the pancreatic islet, ductular epithelial, and centro-acinar cells. Nevertheless, this lesion of the pancreas would be more distinctively

designated "glycogen infiltration," in keeping with customary usage for pathologic accumulations of glycogen in other tissues.

Although so-called hydropic degeneration in the pancreas has been accorded unique status as the histopathologic common denominator of human and experimental diabetes ever since Allen [3] first produced the experimental lesion, certain recognition of its presence in diabetic human material has always posed a difficult diagnostic problem. Post-mortem autolytic phenomena may produce simulated vacuolar appearances in islet cytoplasm; on the other hand, shrinkage referable to fixation artifact may so distort cytologic detail that actual vacuolar effects may become obscured. Warren [18] found the incidence of the lesion to be no greater in material from the pre-insulin era than in that obtained subsequently. However, in experimental alloxan diabetes of rabbits, provision of exogenous insulin, even in amounts insufficient to affect appreciably the level of hyperglycemia and causing only moderate diminution of glycosuria, may prevent the development of the lesion or restore affected cells to normal structural appearance.[22] Thus, apparently anomalous observations on routinely prepared human material are to be expected. Weichselbaum [19] reported vacuolated islet cells in 67 of 183 pancreases of diabetic patients (36.6 per cent), whereas Warren [18] found them in only 22 instances among 484 autopsies (4.5 per cent). As reported by various authors,[31] the incidence has varied from 48 per cent to zero. The true incidence of the lesion in human material is probably insusceptible to exact analysis. However, utilization of histologic technics for demonstrating glycogen in suspected lesions should permit an improved accuracy of diagnosis. The presence of histologically demonstrable glycogen in human islets apparently affected by characteristic cytoplasmic vacuolation constitutes positive morphologic evidence of diabetes mellitus. Failure to find glycogen in characteristically vacuolar islet cytoplasm need not necessarily mean the absence of diabetes; such failure might result also from either post-mortem glycolysis or from imperfections of fixation and staining.

The significance of accumulation of glycogen in various pancreatic cells remains obscure. Possibly it represents merely one component of the more widespread pathologic deposits found in subjects whose diabetes has been poorly controlled. One alternate explanation which may be suggested tentatively is that in the diabetic pancreas, glycogen infiltration may be an indication of deranged regeneration of islets. This idea derives from the occurrence of the material in the islet cells,

centro-acinar cells, and ductular epithelium. These latter cells are the principal histogenetic source of differentiated islet cells in fetal and probably also in extra-uterine life. Furthermore, the pancreatic duct epithelium of the human fetus has been reported to contain glycogen [17] and it is known that embryonic tissues in general contain more chemically determinable glycogen than their mature derivatives. On the other hand, mitotic activity as an indicator of proliferative regeneration is not a common feature of diabetic pancreases showing glycogen infiltration except in animals treated with anterior pituitary extract.

A second alternative is suggested by the renal lesion known as glycogen nephrosis. Renal tubular epithelial cells normally resorb and transmit glucose from the glomerular filtrate, but they do not normally contain histologically demonstrable glycogen. When their functional capacity to handle glucose is exceeded, as in severe spontaneous or experimental diabetes mellitus and in phloridzin diabetes, these renal cells accumulate glycogen in their cytoplasm. Allen [3,20] considered the pancreatic islet lesion a regressive morphologic response to prolonged excessive functional stimulation; because of its presumed aqueous character and for want of analogous phenomena in other organs or tissue, he thought so-called hydropic degeneration was unique. The demonstration of glycogen in pancreatic cells in diabetes establishes a degree of parallelism between the morphologic response of the overstrained pancreatic islets and the renal tubular cells. However, a normal physiologic process provides a basis for the development of the renal lesion, whereas no analogous pancreatic function has been determined.

Fixation in Helly's fluid offers several advantages for the histologic demonstration of glycogen infiltration in the pancreas. As with other chrome-containing solutions, comparable amounts of glycogen are found in the central and in the peripheral regions of blocks fixed in Helly's fluid. Use of celloidin for embedding, or application of a celloidin film to paraffin-embedded sections mounted on slides is unnecessary. Furthermore, such fixation of pancreatic tissue not only permits utilization of methods recommended for histologic demonstration of glycogen and enzymatic digestion of control preparations, but also provides excellent material for concurrent assessment of islet cytoarchitecture with respect to cytoplasmic granulation and relative numbers of cell types. Although the periodic acid-Schiff's reagent routine colors many substances other than glycogen, its reproducibility, simplicity, and brilliant differentiation make it especially valuable for screening old material of doubtful technical quality. Confusion of glycogen with mucin, fibrin, hyalin, basement membranes, and other

substances colored by the periodic acid-Schiff's reagent reaction can be circumvented, at least partially, by enzymatic digestion of control sections. Even so, anomalous results occur with all technics employed in this study; therefore, careful utilization of sections known to contain glycogen as controls for both the staining and digesting processes is essential to accurate interpretation.

Summary

In so-called hydropic degeneration of the pancreatic islets and ductular epithelium of experimental and human diabetes, glycogen was demonstrated in the vacuolated cells by common histologic technics.

The characteristic vacuolar appearance of swollen pancreatic cells affected by "hydropic degeneration" was shown to be referable to artifactual removal of intracytoplasmic accumulations of glycogen rather than to excessive quantities of water or serous fluid.

This pancreatic lesion would be more precisely and distinctively designated by the term glycogen infiltration.

Failure to demonstrate glycogen in suggestively vacuolated islet cells does not necessarily preclude the possibility of its having been present at the time of death.

Glycogen infiltration of human islets of Langerhans may constitute positive morphologic evidence of diabetes mellitus.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 56

Figs. 1 to 6. The swollen, vacuolar appearance of various types of cells after artifactual removal of glycogen from their cytoplasm by routine histologic methods.

FIG. 1. Human vaginal epithelium. Formalin fixation; hematoxylin and eosin stain. × 530.

FIG. 2. Human parathyroid adenoma. Formalin fixation; hematoxylin and eosin stain. × 530.

FIG. 3. Human heart muscle fibers adjacent to an area of infarction. Formalin fixation; hematoxylin and eosin stain. × 530.

FIG. 4. Armanni lesion in renal tubular epithelium of the rabbit in permanent, severe alloxan diabetes. Helly's fluid; hematin, phloxine, and saffron method. × 530.

FIG. 5. So-called hydropic degeneration in the pancreatic islet and ductule (lower right) of the rabbit in permanent, severe alloxan diabetes. Helly's fluid; modified Gomori's chromic alum hematoxylin stain. × 530.

FIG. 6. Human liver in von Gierke's disease. Formalin fixation; hematoxylin and eosin stain. × 530.

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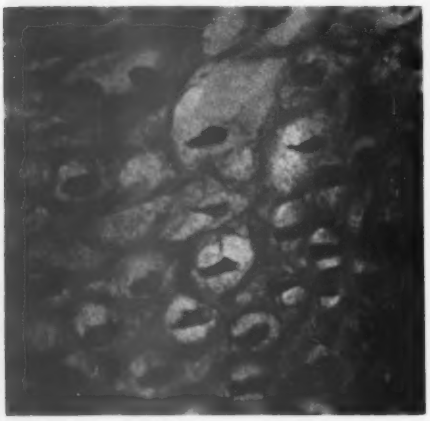

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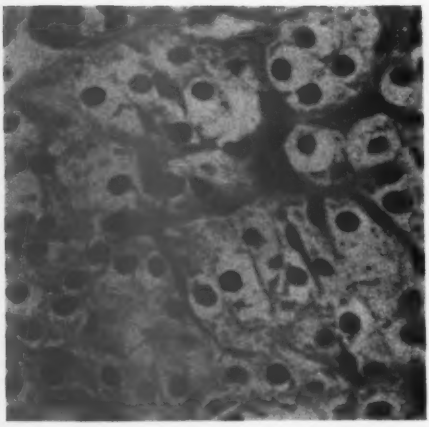

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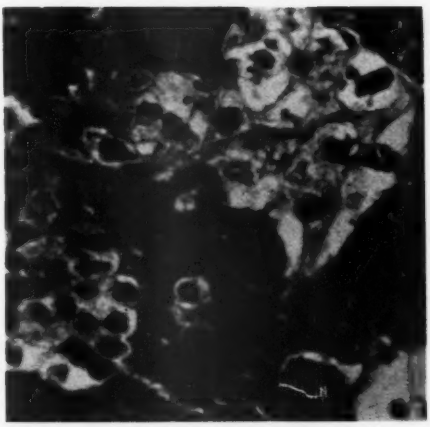

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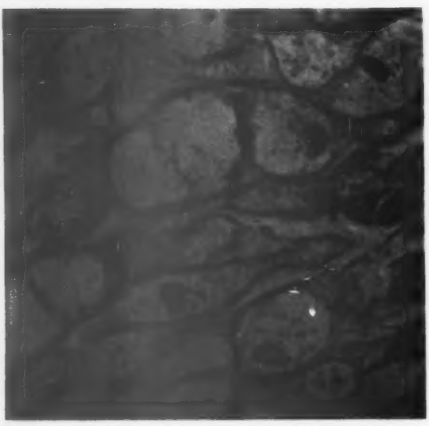

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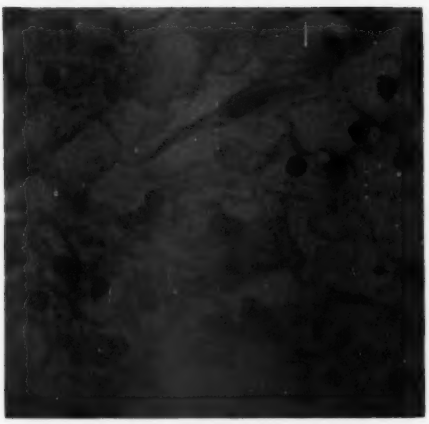

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Toreson

Glycogen Infiltration in the Pancreas

PLATE 57

Figs. 7 to 9. Artifactual ipsilateral displacement of histologically demonstrated glycogen.

FIG. 7. Human vaginal epithelium. Formalin fixation; Best's carmine stain. × 530.

FIG. 8. Human "hydropic" heart muscle fibers adjacent to an area of infarction. Formalin fixation; Bauer's chromic acid-Schiff's reagent. × 530.

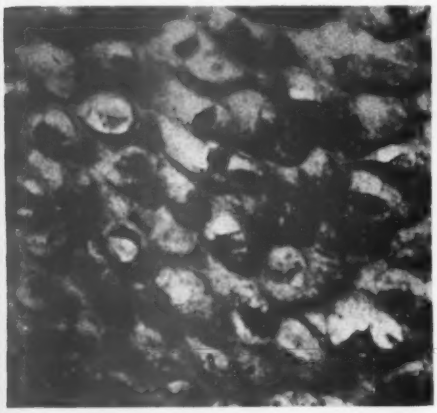
FIG. 9. Armanni lesion in the renal tubular epithelium of the rabbit in alloxan diabetes. Helly's fluid; Hotchkiss' alcoholic periodic acid-Schiff's reagent. × 530.

Figs. 10 to 12. Pancreas of alloxan diabetic rabbit.

FIG. 10. Glycogen in pancreatic islet cells and centro-acinar cell (lower right). Helly's fluid; Best's carmine stain. × 530.

FIG. 11. Removal of glycogen from pancreatic islet cells and ductule (upper right) by diastase. Helly's fluid; diastase; Hotchkiss' alcoholic periodic acid-Schiff's reagent. × 530.

FIG. 12. Absence of demonstrable mucin from vacuolated pancreatic islet cells. Helly's fluid; Mayer's mucicarmine stain. × 530.



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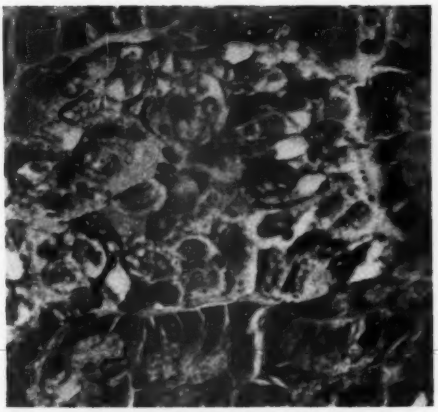

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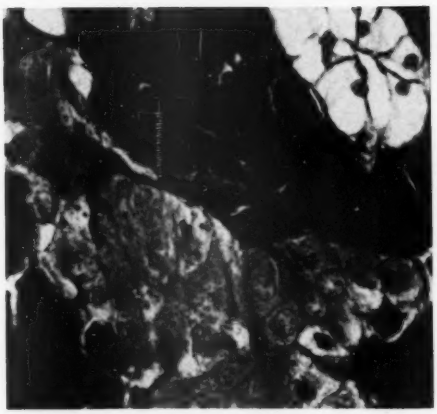

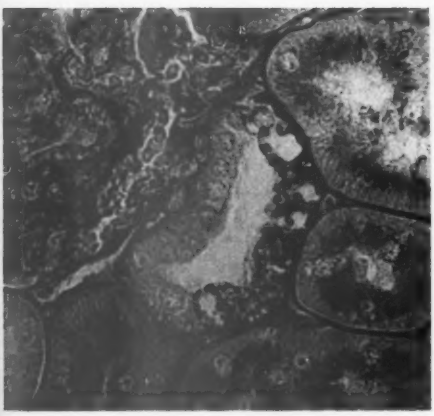

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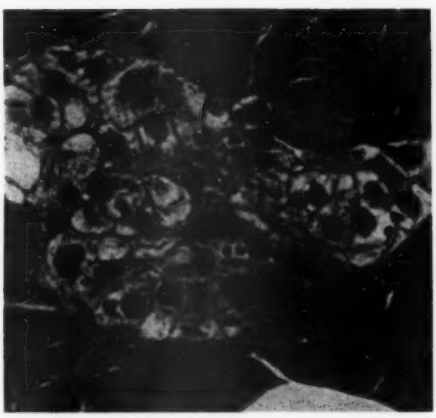

Toreson

Glycogen Infiltration in the Pancreas

PLATE 58

Figs. 13 to 15. Pancreas of a dog treated with anterior pituitary extract.

FIG. 13. Extensive degranulation and slight vacuolation of beta cells of islet. Helly's fluid; modified Gomori's chromic alum hematoxylin stain. × 530.

FIG. 14. Small crescentic bands of glycogen in a few islet cells. Helly's fluid; Best's carmine stain. × 530.

FIG. 15. Removal of glycogen from an islet and ductule by diastase. Helly's fluid; diastase; Best's carmine stain. × 530.

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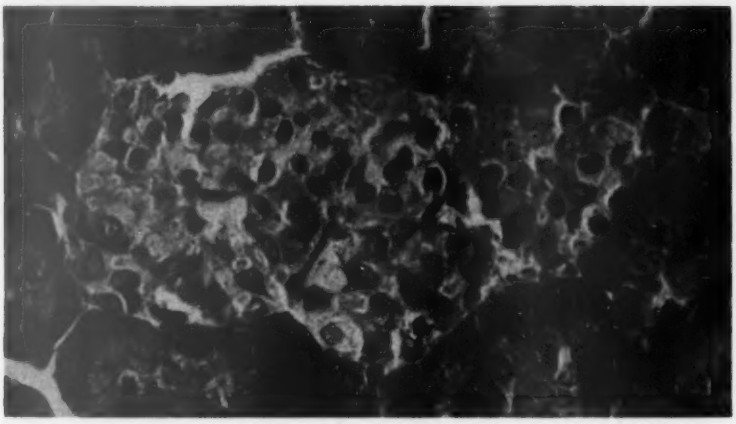

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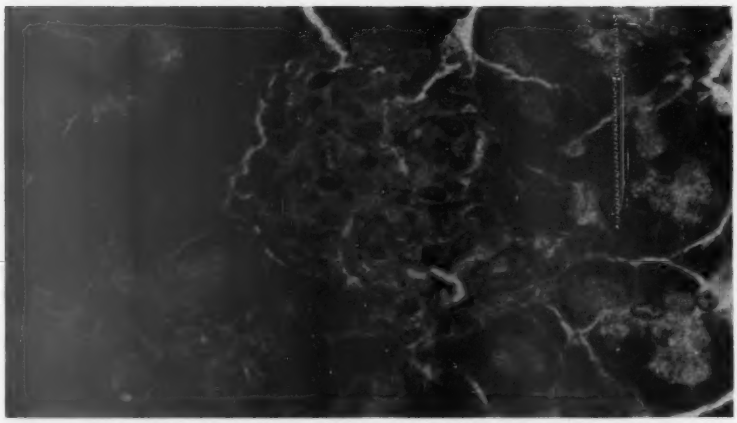

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Toreson Glycogen Infiltration in the Pancreas

PLATE 59

Figs. 16 to 18. Pancreas of cortisone-treated rabbit.

FIG. 16. Marked swelling and vacuolation of beta islet cells without regressive nuclear changes. Vacuolation of centro-acinar cells. Zenker's formal fixation; Masson's trichrome stain. × 530.

FIG. 17. Abundant quantities of glycogen in the cytoplasm of beta islet cells. Several islet and centro-acinar cells incompletely filled by peripherally displaced glycogen. Zenker's formol fixation; Best's carmine stain. × 530.

FIG. 18. Removal of glycogen from beta islet cells by diastase. Zenker's formol fixation; diastase; Best's carmine stain. × 530.

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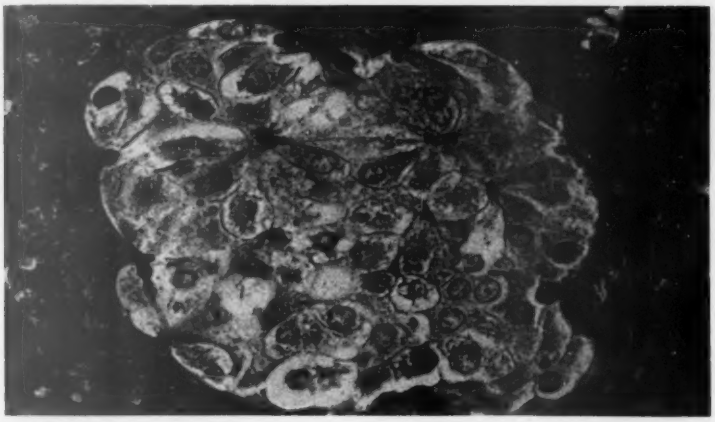

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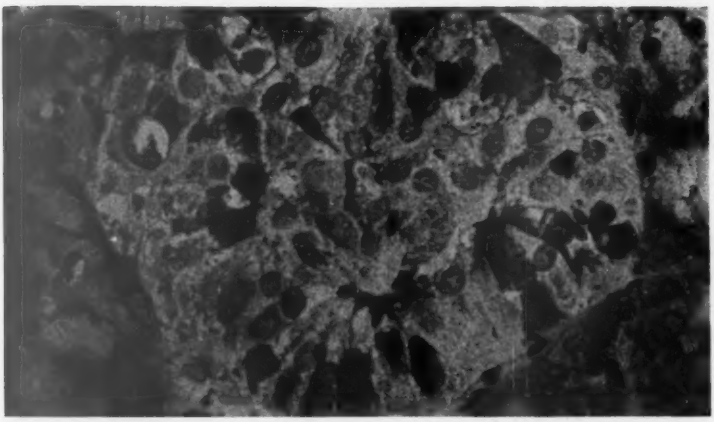

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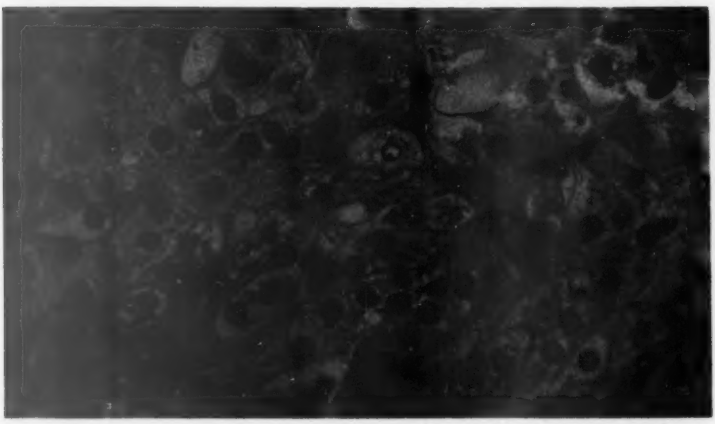

Toreson Glycogen Infiltration in the Pancreas

PLATE 60

Figs. 19 to 21. Pancreas of a patient who died in diabetic coma.

FIG. 19. Swollen and vacuolated beta islet cells. Acetic acid-Bouin's fixation; Masson's trichrome stain. × 530.

FIG. 20. Widespread deposition of granules of glycogen in an islet. Acetic acid-Bouin's fixation; Best's carmine stain. × 530.

FIG. 21. Removal of glycogen from an islet by diastase. Acetic acid-Bouin's fixation; diastase; Best's carmine stain. × 530.

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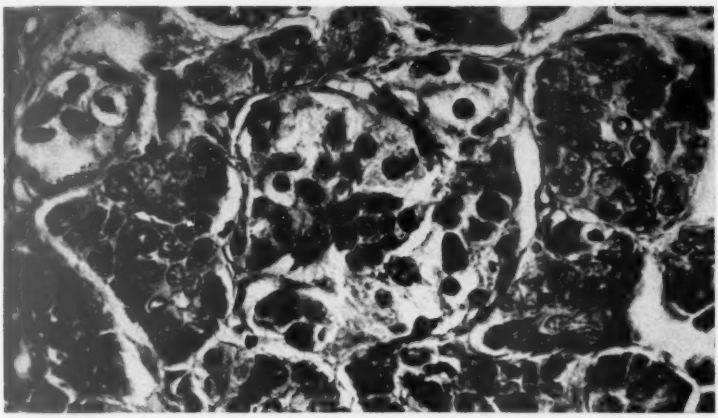

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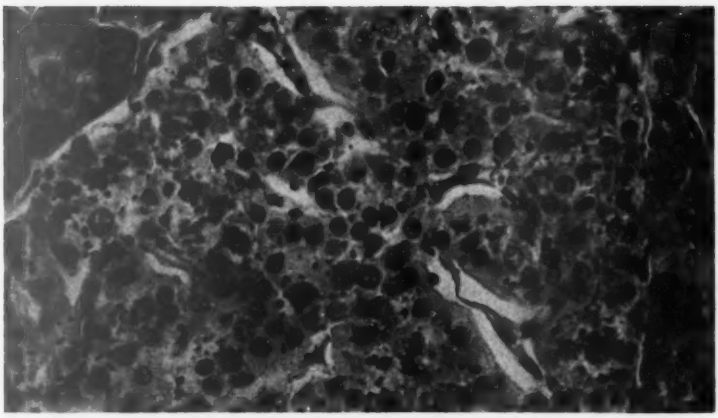

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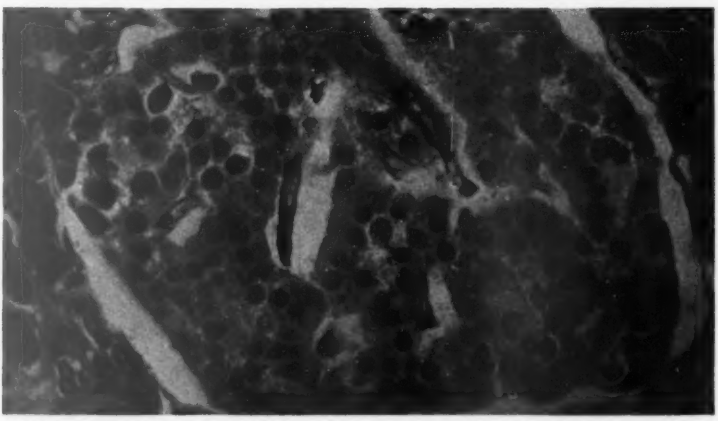

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